THE EFFECT OF DIETARY CATION-ANION BALANCE ON MINERAL BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

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Bachelor of Science

Texas A&M University

College Station, Texas

1988

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1992

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ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Don Topliff and Dr. David Freeman for their continued support and advice during my research project. I would also like to acknowledge Dr. Jim Breazile and Dr. Don Wagner who served on my committee. Also, I would like to thank Dr. Bill Tucker who generously supplied much of the necessary lab equipment and expertise.

I would also like to thank Ward Stutz who worked on a companion project and served as lab technician for making the past year enjoyable and rewarding.

I would like to acknowlege Stacy Lee, Equine Herd Manager, for assisting during the trial. Stacy provided us with many tips from his past research experience that made our work much easier and safer for all those involved.

Maria Mottola, FeFe Melouk, Jackie Cherry and Donna Perry were very instrumental in the success of my lab work. Not only did they provide their much needed expertise and overtaxed time and patience but also the use of their lab equipment and supplies. To these four ladies, I am deeply in debt and will probably remain so throughout my graduate career.

Many thanks and much more acknowledgment is certainly due the students who showed up on time every morning at 6am and worked frantically until the last possible minute before time for class. Without this support crew the project would not have been possible; Trigg Rentfro, Lance Baker, George Hampton, Becky Phelps and Gina Phipps. Again, I would like to extend my sincere appreciation to all these people.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Much of the current research on the nutrient requirements of the exercising horse has been focused on energy and protein and led to improved ration formulation for performance horses. The area of mineral requirements has only recently received attention. Current recommendation on mineral requirements for most classes of horses are not well defined, and research on the effect of exercise on mineral requirements is fragmented and incomplete. It is suspected that exercise influences mineral metabolism as evidenced by changes in circulating concentrations of calcium, phosphorus, potassium, chloride and sodium. Also, it is known that significant amounts of minerals are lost in sweat. Potassium, the major intracellular ion, is involved in the maintenance of acid-base balance and osmotic pressure. Sodium, the major extracellular ion, is also involved in acid-base balance and osmotic regulation of body fluids. Chloride normally accompanies sodium and is also involved in acid-base balance and osmotic regulation of body fluids. These three ions are the main components of the equation used to express dietary cation-anion balance (DCAB). Research data from other species indicates that the acid or base producing power of a diet has significant effects on calcium and magnesium balance, blood and urine pH, milk production, adaptation to heat stress, and the occurrence of developmental bone diseases. However, little or no work has been conducted on the effects of DCAB, calculated as meq((Na + K) - Cl)/kg diet DM, on mineral metabolism in the exercising horse. Diets fed to

most exercising horses have calculated DCAB near 150 meq/kg of dry matter and may be as low as 50-100 meq/kg dry matter. Those levels would be considered as marginal to deficient for poultry and dairy cattle rations in terms of maintaining optimum blood pH and calcium retention. While short term effects of feeding such diets may or may not be noticeable, long term effects could significantly influence the health and performance of horses. If manipulating the DCAB could be shown to improve calcium retention or balance, there is potential for minimizing skeletal demineralizations and the associated changes in bone strength. Also, if DCAB could be shown to elevate blood pH or prevent the depression of blood pH associated with anaerobic exercise, the onset of fatigue due to metabolic acidosis might be delayed and ultimately performance improved.

Research on the effects of DCAB on mineral balance is needed if owners and trainers are to maximize performance and minimize the health risks to exercising horses. Therefore, the objectives of this trial were to investigate the effects of dietary cation-anion balance on mineral balance, blood pH, and urine pH in the anaerobically exercised horse.

CHAPTER II

LITERATURE REVIEW

Evolution of Dietary Cation-Anion Balance

The dietary electrolytes sodium, potassium, and chloride have received minimal attention in animal nutrition research. This might partially be explained by the rare occurance of electrolyte deficiencies in common animal rations. Diets usually contain potassium in excess of most animal requirements, and sodium and chloride may be easily supplemented as salt. However, the ratio of these ions in the composition of ration ingredients or within supplemental form is usually not considered. In recent years, it has become apparent that the interrelationships between these elements play an important role in nutrition and should be more precisely controlled and studied.

Mongin (1980) was one of the first researchers to propose a balance equation which included the dietary electrolytes sodium (Na), potassium (K), and chloride (Cl) as follows: meq (Na + K) - Cl/100g diet dry matter. The equation was used to express a diet's ability to affect acid-base physiology. More recent equations have included all ionizable elements in the diet regardless of their valence state or availability. However, Mongin's equation is still the most commonly used and considers only the monovalent elements Na, K, and Cl as they are the most readily absorbed from the gut and appear to have the greatest effect on the acid or base producing power of the diet (Austic, 1988). The units of this equation are appropriately in milliequivalents instead of milligrams as

these elements produce their physiological effects according to their valence rather than their weight.

Either dietary cation-anion balance (DCAB) or acid-base balance has been the accepted terminology used to describe the interrelationships of these electrolytes. However, since the DCAB exerts its physiological effects via the acid-base system of the body, it has in recent years become the most popular and printed term.

Equine Studies

Mineral Requirements

Sodium. The NRC lists the sodium concentrations of common feedstuffs to be less than 0.01 in many cases. Therefore, sodium is routinely added to horse rations or provided in supplemental form. Although sodium is the major extracellular cation and the major electrolyte involved in the maintanence of acid-base balance and osmotic regulation of body fluids, there are no reported studies defining the sodium requirement based on Mcal of digestible energy intake per day. However, an optimum sodium concentration for equine diets has been reported to range between 1.6 and 1.8 g/kg of dry matter for maintenance, growth, and late gestation (Jarrige and Martin-Rosset, 1981). The same study reported a two fold increase in sodium requirement to 3.6 g/kg of dry matter for horses performing moderate to heavy work. Drepper et al. (1982) reported a maintenance requirement of 15 g/day and a light to heavy work requirement of 21 to 36 g/day for a mature 600 kg horse. Generally, the sodium requirement for work is reported as roughly twice that for maintenance. However, the requirement may dramatically increase when exposed to

prolonged exercise or elevated temperatures as sodium is a primary component of sweat. Sweat sodium losses have been reported to range between 8.25 to 82.5 g depending on the level of exercise (Meyer, 1987). More recently, Young et al. (1989) reported sodium losses in sweat of 42.1 mg/kg of body weight in miniature horses during exercise at a work load of 945 kg·km. Based on the limited data reported on sodium requirement, the 1989 National Research Council Nutrient Requirements of Horses (NRC) lists the adequate concentration of sodium for maintenance, pregnant and lactating mares, and growth as 0.10 percent of the total ration on a dry matter basis. The requirement for working horses is listed at 0.30 percent of the total ration (DM).

Potassium. The NRC (1989) lists the potassium concentration of forages and oilseed meals as 1 to 2 percent on a dry matter basis. The common cereal grains corn, oats, and wheat contain 0.3 to 0.4 percent potassium. Therefore, the potassium requirement is easily met as forages represent a large proportion of horse rations,. Hintz and Schryver (1976) reported mature horses required 0.06 g of potassium/kgBW/d or approximately 0.4 percent of the diet. In 1981, Jarrige and Martin-Rosset reported an optimum potassium concentration for light to medium work of 0.4 to 0.5 percent. Drepper et al. (1982) estimated the potassium maintenance requirement of a 600 kg horse to be 22 g/d. The requirement was estimated to increase to 32 g/d for light work, 43 g/d for medium work, and 53 g/d for heavy work. Meyer et al. (1985) suggested that sweat losses of potassium are so large that the requirement for the heavily exercised horse may be twice that of the sedentary horse. Young et al. (1989) reported sweat potassium losses of 138.1 mg/kg BW in miniature horses exercising at a work load of 945 kg·km. Based on the previous information, the NRC (1989) estimates the potassium requirement for maintenance to be 0.05

g/kg BW or 1.52 g/Mcal of DE. The NRC further suggests an increase in the potassium requirements of 1.1, 1.4, and 1.8 times maintenance for light, medium, and heavy work respectively, based on the work of Drepper et al. (1982).

Chloride. Chloride is also an essential component of gastric secretions necessary for digestion and is an important extracellular anion involved in acid-base balance and osmotic regulation of body fluids. However, the chloride concentrations of feedstuffs are not well defined, and the requirements of horses have not been established. Nonetheless, chloride requirements are assumed to be met when sodium requirements are met with the addition of sodium chloride to the diet.

Magnesium. The NRC (1989) states the magnesium concentrations of common feedstuffs ranges from 0.1 to 0.3 percent. Hintz and Schryver (1972) and Meyer (1979) reported a range from 40 to 60 percent for the true absorption efficiency of magnesium. McKenzie (1981) reported magnesium to be 42 to 45 percent digestible. Drepper et al. (1982) proposed a daily magnesium requirement of 12 g for maintenance of a 600 kg horse and that the requirement increases by 1 to 2 g/day for light to medium work. Given a true absorption efficiency of 40 percent, the NRC (1989) suggests a magnesium requirement of 15 mg/kg BW/day or .46 g/Mcal of DE/day.

Sulfur. To date, sulfur has received very little attention from equine nutrition researchers, and no data has been reported on sulfur requirements in the exercising horse. It is known that nonruminant animals meet their sulfur amino acid requirement primarily from organic sulfur forms including cystine and methionine. Some dietary inorganic sulfur is incorporated into sulfur-containing

microbial protein in the hind gut of the horse. However, there is limited amino acid absorption from this region. High quality dietary protein sources usually provide a minimum of .15 percent organic sulfur on a dry matter basis. Based on the work of Jarrige and Martin-Rosset (1981), the NRC (1989), suggests that this level is adequate in meeting the sulfur requirement of all classes of horses.

Phosphorus. The estimated efficiency of true phosphorus absorption ranges from 30 to 55 percent in the horse (NRC, 1989). The variation is due to age of animal and the source and concentration of phosphorus in the diet (NRC, 1989). Because idle horses, gestating mares, and working horses consume mainly plant sources of phosphorus, the NRC (1989) suggests a true absorptive efficiency value of 35 percent. The value is increased to 45 percent for lactating mares and growing horses as their diets are supplemented with inorganic forms of phosphorus. Given these values, the NRC (1989) list the phosphorus maintenance requirement at 28.6 mg/kg BW/day or 0.87 g/Mcal of DE/day.

Calcium. The NRC (1989) suggests the true absorptive efficiency of calcium ranges from 70 percent in young horses to 50 percent in mature horses. However, for the purposes of estimating calcium requirements, the NRC (1989) suggest a value of 50 percent be used for the calcium absorptive efficiency for all classes of horses. Using this value, the calcium requirement is stated to be 0.04 g/kg BW/day for maintenance or 1.22 g/Mcal of DE/day. The NRC (1989) suggest that any increase in calcium requirements associated with exercise should be met by the obligatory increase in calcium intake as dry matter consumption increases to meet energy demands.

Mineral Studies

In 1970, Schryver et al. investigated the effect of level of calcium intake on skeletal metabolism and the homeostatic mechanisms of calcium metabolism in young growing ponies. Dietary calcium levels below (0.15%), equal to (0.8%), and greater than (1.5%) that suggested by the NRC (1966) were formulated by adding calcium carbonate at the expense of hay and corn. These variations in calcium intake produced large differences in excretion and retention of calcium in order to maintain calcium homeostasis but had no effect on the level in the plasma or on the size of the exchangeable pool. Ponies fed the low calcium diet responded with increased fractional absorption of calcium while decreasing the renal excretion rate. Also, the removal rate from bone was increased to a level which exceeded the deposition rate producing a net transfer of calcium from bone into the exchangeable pool. Nonetheless, these ponies experienced negative calcium balance in spite of the homeostatic control mechanisms. Opposite responses were observed when the ponies were fed the high calcium diet. However, unlike rate of removal, the depostion rate was insensitive to the level of calcium in the diet.

In a following study, Schryver and coworkers (1971a) looked at the effect of high dietary phosphorus levels on calcium utilization and skeletal metabolism in growing Shetland ponies fed diets containing 0.4% calcium and either 0.2% or 1.2% phosphorus. As expected, the phosphorus retention and plasma levels were increased when the ponies were fed the high phosphate diet. However, calcium absorption, renal excretion, and retention were each decreased while total and endogenous fecal calcium excretion increased. Schryver et al. (1971b) demonstrated that renal phosphorus excretion, total amount of phosphorus absorbed from the gut, and retention were all dependent upon phosphorus

intake. However, the efficiency of absorption was unaffected by the level of phosphorus in the diet and averaged 45 percent.

Williamson (1974) evaluated the serum electrolyte (Na, K, Cl) and serum HCO3 levels from Thoroughbred and Standardbred horses experiencing poor race track performance. Control values for these parameters were obtained from 200 individual winners. Various forms of alkalosis and acidosis were associated with elevations or depressions in electrolyte and bicarbonate levels and/or combinations thereof. He also reported that these disorders could be treated by supplementing the diet, drenching, or intravenous administration of electrolytes. Thus, electrolyte therapy was proposed as a means to treat acidosis and alkalosis and possibly improve race track performance in horses.

In 1974, Milne studied the effects of exercise on blood parameters, acid-base balance, and electrolyte levels. He demonstrated that acid-base balance was not affected by moderate work below the anaerobic threshold whereas a heavy workload produced a partially compensated metabolic acidosis. These effects on acid-base balance were still present at fifteen minutes post-exercise but were returning toward normal levels. He also proposed a linear relationship between the changes in arterial and venous pH, pCO2, and HCO3 in response to exercise. He also stated that these arterial blood parameters could be accurately predicted from venous samples; however, the pO2 level could not be predicted from these venous samples. Heavy, short-duration exercise produced significant increases in serum calcium most likely due to a shift of calcium out of the muscle cells as a result of the acidosis whereas serum calcium will generally decrease during endurance test. Serum potassium concentration in man has been shown to increase following both short duration exercise and marathon running (Pugh et al., 1967 and Rose et al., 1970). This rise in serum potassium may be from: 1) an influx of potassium from the intracellular stores being

exchanged for hydrogen ions during metabolic acidosis, 2) the diffusion of potassium from the intracellular space when muscle and liver glycogen is reduced, or 3) intravascular hemolysis (Bergstorm et al., 1971; Gilligan et al., 1943 and Kjellmer, 1965). However, Milne (1974) reported no significant change in serum potassium concentration in endurance horses possibly due to the large amount of potassium lost in sweat.

In 1978, Schryver and coworkers investigated the effects of exercise on calcium metabolism, skeletal mass, and the dermal excretion of calcium and phosphorus in yearling Standardbred horses. During exercise periods, the urinary excretion of ^{40}Ca and ^{47}Ca decreased 50 to 75 percent. Although the retention of ^{47}Ca increased during the exercise periods, dietary ^{40}Ca retention did not change. Exercise did not affect the efficiency of absorption or the endogenous fecal excretion of calcium. In a second experiment, they observed no marked differences in relative weight or specific gravity in limb bones of exercised versus non-exercised Shetland ponies. Thus, they concluded that although exercise increases the rate of bone turnover in growing ponies and horses it does not affect the skeletal mass. In the last portion of the experiment, they reported polo horses excreted 80 to 145 mg of calcium and 11 to 17 mg of phosphorus during a twenty-minute workout.

Schryver and coworkers (1987) evaluated the range of voluntary salt (NaCl) intake in horses and its effect on mineral metabolism. The mean daily salt consumption of mature unexercised horses was 53 g with a range from 19 to 143 g. The consumption pattern was not affected by the season of the year mainly because they were sedentary animals with minimal sweat loss. In the metabolism studies, diets containing 1, 3, and 5% NaCl showed that urinary excretion was the primary pathway for sodium and chloride loss. Urinary sodium excretion was directly related to intake with 69 to 78% of the sodium

intake being excreted via the urine at each level of intake. The varying levels of salt intake did not affect fecal excretion, intestinal absorption, or retention of sodium. Chloride was not detected in the feces at any level of salt intake thus indicating that dietary chloride was completely absorbed and that urinary excretion was the sole pathway for chloride excretion. Although urinary calcium and phosphorus excretion was not affected by the level of salt intake, the absorption and retention of calcium and phosphorus were significantly increased when the ponies were fed the 3 or 5% NaCl diets. Magnesium metabolism was unaffected by the level of salt intake.

Young and coworkers (1989) evaluated the extent of mineral losses (fecal, urine, and sweat) in miniature horses at rest and during extensive physical exercise. The total daily feed intake at rest of 1.6 kg and 4.32 Mcal increased to 3.0 kg and 7.93 Mcal/d during the exercise period. As daily sodium intake increased from 7.5 to 25.4 mg/kg BW during exercise period, there was a trend for daily fecal excretion to increase from 1.2 to 8.2 mg/kg BW. Also, daily urinary sodium excretion tended to decrease from 14.6 4.7 mg/kg BW. This is probably due to the large amount of sodium lost in sweat. Daily retention of sodium decreased from -8.3 to -29.6 mg/kg BW during the exercise portion of the trial. This indicates the increase in sodium intake due to the increased feed intake associated with the exercise was not sufficient to compensate for the large amount of sodium lost in heavily exercised horses. This agrees with Meyer (1987) who suggested that most diets contain inadequate sodium concentrations to meet the needs of the exercising horse.

In the same study, the daily chloride intake increased from 25.4 to 40.9 mg/kg BW during the exercise trial. Contradictory to Schryver et al., (1987) fecal chloride increased from 6.3 to 18.9 mg/kg BW. Also, there was a trend for urinary chloride excretion to decrease from 9.0 to 0.9 mg/kg BW. Daily chloride

retention decreased from 10.1 to -69.2 mg/kg BW during the exercise trial primarily due to the large amount of chloride lost in sweat. This disagrees with Meyer (1987) who suggested that the obligatory increase in chloride intake due to the increase in total feed intake in response to exercise to be an adequate supply of chloride.

Young and coworkers (1989) also reported daily intake of potassium increased from 54.9 to 220.2 mg/kg BW in response to exercise. Daily fecal potassium excretion increased from 16.9 to 55.7 mg/kg BW during exercise. Also, daily urinary excretion increased from .3 to 31.6 mg/kg BW. Daily retention of potassium decreased from 37.7 to -5.2 mg/kg BW in response to exercise. Although slightly negative, this agrees with Meyer (1987) suggesting that the increase of potassium due to increased feed intake is sufficient in preventing large potassium deficiencies associated with sweating.

In the same study, the daily intake of calcium increased with exercise from 9.1 to 39.6 mg/kg BW. Accordingly, daily fecal excretion increased from 9.8 to 19.6 mg/kg BW. However, daily urinary excretion decreased with exercise from .6 to .1 mg/kg BW. Daily retention of calcium increased with exercise from -1.3 to 19.7 mg/kg BW. This indicates that the increase in calcium associated with the increase in energy requirement was sufficient in meeting the calcium requirement of these horses.

Young and coworkers (1989) reported daily intake of phosphorus increased from 32.9 to 53.9 mg/kg BW during exercise. The daily fecal excretion increased from 8.5 to 27.3 mg/kg BW and urine fell from 1.6 to 1.1 mg/kg BW. The daily retention of phosphorus increased from 22.8 to 25.2 mg/kg BW thus indicating the increase in phosphorus along with increased DE intake was more than sufficient in meeting the needs of the animals.

DCAB

The equation we used to express dietary cation-anion balance (DCAB) includes the dietary electrolytes sodium (Na), potassium (K), and chloride (Cl) as follows: meq (Na + K) - Cl/kg of diet dry matter. The issue of dietary cation-anion balance is not even addressed in the NRC (1989) and in fact, only within the last couple of years has DCAB begun to receive attention from equine researchers. In 1989, Topliff and coworkers studied the effect of DCAB on mineral metabolism in horses galloped two miles daily at approximately 7 m/sec. No change in heart rate or serum chloride and calcium concentrations were observed. However, he demonstrated a significant increase of calcium in the urine from 9.2 mg/dl of horses consuming higher DCAB diet (150 meq/kg) to 84.7 mg/dl in the urine of horses consuming a low DCAB diet (6.5 meq/kg). Since total urine output was not significantly different, those horses consuming the low DCAB diet excreted more total calcium per day. These horses also had higher concentrations of chloride in the urine (176.1 meq/l vs. 124.8 meq/l). This effect was attributed to the acid producing power of the diet. One could assume that those horses consuming diets of lower DCAB may be in negative calcium balance. If this situation is prolonged, an osteoporotic weakening of the skeletal system as seen in other species might occur.

Lawrence and coworkers (1987) orally dosed racing standardbreds with sodium bicarbonate in a switchback designed experiment and found a higher blood pH post racing even though blood lactate levels were similar to the control. Most of the horses also ran a faster time after the sodium bicarbonate infusion, suggesting a link between maintenance of blood buffering capacity and performance. In a more recent study, Lawerence and coworkers (1990) examined the effect of bicarbonate administration in Standardbreds. They

reported improved race times in those horses treated with $NaHCO_3$ 2.5 hours before the workout. The $NaHCO_3$ treatment increased blood lactate removal which may enhance exercise by postponing the onset of fatigue caused by intramuscular acidosis.

Sodium and potassium are frequently absorbed from the gastrointestinal tract in exchange for the secretion of a proton, namely hydrogen. Also, as chloride is absorbed from the gastrointestinal tract, a bicarbonate ion is secreted. Therefore, any alterations in the amounts of sodium, potassium, or chloride absorbed could be expected to alter the acid or base producing power of that diet and interfere with the normal acid-base status of the animal. However, Kelso et al. (1987) reported conflicting results suggesting that sodium bicarbonate supplementation provided little if any physiological advantage to the exercising horse.

DCAB Effect on Other Species

Rabbit

In most literature today, poultry researchers are credited with being the first to recognize and study the effects of dietary cation-anion balance on physiological and production parameters. However, Morgen and Berger (1915) reported that rabbits fed sodium carbonate had increased bone mineral content compared to rabbits fed sodium chloride. They suggested that the carbonate salt increased the alkaline reserve. From this hypothesis, they also suggested that manipulating the cation-anion balance could induce deficiencies in calcium, sodium, potassium, and magnesium.

Thacker (1959) evaluated a depression in growth and failure to maintain normal blood hemoglobin and bone ash content reported in a previous experiment in rabbits fed a basal diet containing timothy hay grown in heavily fertilized soils. He reported that these abnormalities could be corrected by supplementing the diet with salts of sodium, potassium, calcium, and magnesium carrying an anion metabolized to CO2 and H2O by the animal. However, salts of these same minerals carrying a chloride or sulfate anions were not effective in correcting the abnormalities. It was suggested that the rabbits in this experiment suffered physiological cation-anion imbalance (acidosis) associated with altered mineral metabolism on the animal. He reported that this mineral imbalance induced deficiencies of calcium and potassium in rabbits fed adequate dietary levels of these minerals. He also suggested that this condition might involve the metabolism of additional cations.

Poultry

Researchers in poultry nutrition where the first major livestock group to recognize and study the effects that dietary cation-anion balance could have on production parameters. Early research was primarily interested in how sodium, potassium, and chloride might affect the nutrition and growth of the animal through there physiological roles in the regulation of osmotic pressure and acid-base balance.

In 1964, Nesheim and coworkers reported chicks fed excesses of dietary chloride or sulfate supplied as glutamic acid hydrochloride, calcium chloride, or calcium sulfate suffered dramatic decreases in growth rate. This effect is the major inferiority of amino acid diets as compared to those containing intact protein. However, this depression in growth rate could be alleviated by

supplying equimolar levels of sodium or potassium supplied with glutamate or carbonate. Growth rate was also depressed by excesses of sodium. Again, this effect could be eliminated by adding equivalent amounts of chloride. High levels of potassium alone were better tolerated by the chick than sodium. In a similar study, Melliere and Forbes (1966) noted maximum growth and weight gain in chicks fed a diet with a cation-anion ratio of 1.2 to 1.8. whereas a ration of 0.6 nearly inhibited growth completely. Excess calcium was not effective in offsetting the depression in growth rate due to excess chloride; however, magnesium did partially overcome the reponse.

Frank and Beger (1965), Howes (1967), Anderson (1967), and Mongin (1968) set off a new wave of acid-base physiology research by reporting that the egg-shell calcification process could be altered through the acid-base balance physiology of the laying hen.

Previously, the ammonium ion was thought to be the acidogenic agent while the bicarbonate ion was considered the alkalogenic agent. However, in 1972, Cohen, Hurwitz, and Bar reported that the inclusion of large amounts of ammonium chloride in the diet leads to metabolic acidosis while additional dietary sodium bicarbonate produces metabolic alkalosis. These results indicate that sodium supplied with several salts except chloride causes alkalosis while chloride added in several salt combinations except with sodium causes acidosis. When sodium and chloride are added in equal amounts, no changes in acid-base parameters were detected. Thus, blood pH and HCO3 levels were a function of the dietary ratio of sodium to chloride and not the total amount of either. It was noted that the actual dietary pH is irrevelant in producing alkalosis or acidosis: calcium chloride with a pH near neutral causes acidosis whereas, an acid salt such as sodium monophosphate causes alkalosis

Cohen and Hurwitz (1974) incorporated potassium into the equation for cation-anion balance and described it as having as alkalogenic effect similar to sodium. These findings demonstrated that sodium and potassium are additive in their effects in offsetting the metabolic acidosis produced by elevated levels of dietary chloride. This agrees with Nesheim et al. (1964), who demonstrated that sodium and potassium supplementation was an effective means of counteracting the depression of growth produced by high levels of chloride in the diet.

In 1980, Mongin was one of the first to suggest a balance equation incorporating sodium, potassium, and chloride as follows: mEq(Na+K)-Cl/100g diet dry matter. This equation could be used to actually quantify the acid-base status of a ration. Mongin also demonstrated the interaction between the acid-base balance of the blood with the cation-anion balance in the diet.

In the same year, Hamilton and Thompson reported decreased blood pH and bicarbonate levels and reduced eggshell strength and thickness in hens when the chloride level was increased from .11 to 2.13% of the diet. This agrees with Hall and Helbacka (1959), Hunt and Aitken (1962), and Saveur and Mongin (1971) who reported excessive levels of acid chlorides depressed eggshell calcification. Similarly, Frank and Burger (1965), Howes (1967), and Mongin (1968) demonstrated an increase in egg shell strength associated with feedstuffs that increase the alkaline reserve.

The cation-anion balance has also been correlated with incidence of tibial dyschondroplasia (TD). Leach and Neshium (1965) described this bone disorder occuring in young chicks. They later discovered that this condition is affected by dietary cation-anion manipulation (Leach and Neshium, 1972). Sauveur and Mongin (1978) reported that metabolic acidosis resulting from excessive dietary chloride intake increased the incidence of tibial

dyschondroplasia. These studies agree with more recent work relating the anionic content of diets with alteration in acid-base status and ultimately the incidence of TD (Edwards, 1984, Halley et al., 1987, Hamilton and Thompson 1980, Hurwitz et al., 1973, Mongin, 1981).

Nelson and coworkers (1981) reported dry matter and amino acid digestion were lower in 30 day-old-chicks fed diets that contained the greatest cation-anion balance manipulated by supplementing with calcium or phosphorus. In a similar study, Riley and Austic (1983) evaluated the effect of dietary electrolytes on the pH of the digestive tract and the acid-base status of the chicken. The cation-anion status was altered by adding either potassium bicarbonate or calcium chloride. They reported the pH of the crop was depressed by dietary chloride. However, neither chloride nor potassium had an effect on the pH of the proventriculus, duodenum, or the middle and distal portions of the small intestine. The addition of dietary chloride did decrease the plasma bicarbonate, base excess, and pCO2 but did not decrease the plasma pH.

Rat and Human

As a result of the recent interest in osteoporosis in humans, DCAB research in rats has been slanted toward the effects of acid-base physiology on bone metabolism. Barzel and Jowsey (1969) reported significant increases in bone resorption in adult rats with long term consumption of ammonium chloride. However, chronic ingestion of sodium and potassium carbonate prevented the loss of bone tissue, apparently by stimulating bone formation. These responses were attributed to the changes in bone metabolism during systemic acid-base

alterations. Therefore, it was suggested that the intracellular mechanism controlling bone deposition and resorption are sensitive to systemic pH.

In 1975, Newell and Beauchene evaluated the effects of acid stress on renal, serum and bone responses in 13 and 25-month-old rats fed ammonium chloride for nine months. The acid-stressed rats showed decreases in urinary pH along with increases in urinary calcium and phosphorus excretions and kidney weights. The acid-stressed rates tended to have decreased serum calcium and phosphorus levels. However, bone analysis in either age group was not affected by acid stress. In a similar study, Petito and Evans (1984) noted increases in urinary calcium in rats fed ammonium chloride. These rats also had a two-fold increase in fecal calcium as well. As a result, femur specific gravity was decreased in the rats fed the more acidic diet. Additional studies support these findings in the rat (Cole and Zlotkin, 1983; Emerick, 1984) and in humans (Walser and Browder, 1959; Lemann and Relman, 1959; Adams et al., 1979; Schuett et al., 1980). Also, rats given salt supplements excreted more calcium in the urine and had less calcium in the bone than control rats (Goulding and Campbell, 1984). Again, similar effects have been reported in humans (Kleeman et al., 1964).

Whiting and Draper (1980) investigated the effects of different levels of sulfur on the hypercalcuria produced by high protein diets. Their results showed a linear relationship between calcium excretion and sulfate excretion. A peak calcium excretion occurred after only two days. Although the level of urinary calcium declined, a moderate hypercalcuria persisted throughout the eight week experiment. Also, the degree of hypercalcuria was proportional to the sulfur content of the diet. It was proposed that the production and excretion of sulfate are the major factors in the hypercalcuria associated with high protein feeding and are dependent upon the sulfur amino acid content.

In a similar human study, Schuette and coworkers (1980) studied the metabolic effects of protein intake on urinary calcium excretion, calcium absorption, and calcium balance in older rats. An increase in protein intake from 47 to 112 g while maintaining mineral levels constant resulted in increased urinary calcium and a decrease in calcium retention. Glomerular filteration rate was increased and fractional renal tubular absorption was decreased when protein intake increased. The changes in urinary calcium were positively correlated with the increase in total renal acid and sulfate excretion and with the decrease in fractional renal tubular reabsorption of calcium.

Dairy Cattle

In 1986, Coppock reviewed the current literature concerning the influence of DCAB on livestock production. At that time, DCAB research in dairy cattle was minimal. However, he was able to calculate and evaluate the DCAB in various beef and dairy trials conducted in the past. He suggested that the ruminant animal could withstand a higher DCAB than poultry. He also stated that manipulating the DCAB from 10 to 40 meq/100 g diet dry matter would yield no beneficial results. Escobosa and coworkers (1984) reported that cows consuming a negative DCAB diet suffered decreased intake. Since that time, dairy researchers have made more progress towards understanding the physiological effects of DCAB than any other specie.

The recent surge of interest in DCAB research in the dairy industry came when Block (1984) examined the effectiveness of a low DCAB diet in preventing the occurrence of parturient paresis in lactating dairy cows. In previous research, a link between dietary anions and increased calcium availability had been established (Dishington, 1975; Ender et al., 1971; Lomba et al., 1978).

Block (1984) demonstrated that highly anionic diets (-128 meq/kg DM) fed during the dry period reduced the incidence of parturient pariesis during lactation. Tucker et al. (1988) showed that linearly altering the DCAB from -100 to +200 meq/kg resulted in a linear increase in both milk production and blood pH. These workers also demonstrated that the effects were attributable to DCAB alone and not due to changes in the absolute amount of sodium, potassium, or chloride in the diet.

Maintaining a constant blood pH is a critical normal body function accomplished by a homeostatic mechanism reponsible for maintaining a constant blood bicarbonate to blood pCO2 ratio. According to Tucker and coworkers (1988), this is accomplished by altering renal excretion of bicarbonate to control blood bicarbonate concentrations and altering respiratory rate to control blood pCO2. Altering the DCAB of the diet has been shown to have marked effects on blood acid-base balance. Diets with low DCAB or high in chloride have been shown to depress blood pH (Tucker, 1988). Tucker reported a linear relationship between blood pH, bicarbonate, and DCAB.

Beighle and coworkers (1990) reported that calves fed diets with low DCAB had higher concentration of serum and fecal phosphorus. These calves also had decreased levels of phosphorus in the bone. These effects were amplified when a low phosphorus diet was fed thus indicating an interaction between DCAB and dietary phosphorus level on changes in blood, fecal, and bone phosphorus concentrations.

Tucker and coworkers (1991) evaluated the influence of supplemental dietary sodium bicarbonate on potassium metabolism of young growing dairy calves. They reported feed intake was not affected by dietary potassium chloride or sodium bicarbonate supplementation. However, average daily gain increased with increased potassium but tended to decrease with increased

dietary sodium bicarbonate. Plasma potassium was elevated by the increased potassium intake. Urinary calcium excretion appeared to decline in response to sodium bicarbonate whereas urine pH increased.

Tucker et al., (1991) also examined the influence of calcium chloride on systemic acid-base status and calcium metabolism in dairy heifers. The plasma free proton concentration increased and bicarbonate decreased with increasing calcium chloride intake. Plasma calcium and urinary hydroxyproline excretion were unaffected. However, renal calcium excretion rose with calcium chloride intake possibly due to increased bone resorption or intestinal absorption of calcium. Plasma and urinary chloride levels increased with increased dietary chloride intake.

Recent research conducted by Tucker et al., (1991) demonstrated that dietary chloride and sulfur had similar effects on the acid-base status of dairy cows. This is in agreement with Oetzel (1991), who analyzed the previous research conducted on acid-base balance in dairy cattle and determined that sulfur is the primary ion affecting acid-base balance. Therefore, they suggest that sulfur be included along with chloride in the DCAB equation for lactating dairy cows.

Parathyroid hormone has been shown to have a more dramatic effect on renal production of 1,25-dihydroxyvitamin D in dairy cows fed highly anionic diets thus increasing intestinal calcium absorption (Goff et al., 1991). Also, osteoclastic bone resorption was more responsive to parathyroid hormone as plasma hydroxyproline concentration was higher in those cows fed the low DCAB diet. The parathyroid hormone activity might be due to the decrease in the pH of the blood, which in dairy cattle and poultry has been shown to be a possible cause for increased levels of ionized or free calcium in blood and

therefore an increase in urinary calcium excretion (Austic , 1984 and Tucker et al., 1988).

Swine

Although the early investigations of DCAB have involved poultry, swine nutritionists have begun to show interest in this area. Yen et al. (1981) indirectly touched on this subject when they observed that the addition of 4% calcium chloride to the diet resulted in reduced feed intake, weight gain, and feed efficiency in crossbred barrows. The diet also caused an increase in plasma chloride and a reduction in blood pH, base excess, and total CO2 and HCO3 indicative of metabolic acidosis. These effects could be reversed and actually elevated by the addition of 2.03% sodium bicarbonate.

Golz and Crenshaw (1984) examined the importance of sodium, potassium, and chloride on growth rate in young pigs. They noted growth was a function of potassium to chloride ratio with the optimum being 2.1 to 1. This effect was independent of sodium as long as sodium was within the range of .03 to .6%.

Patience and coworkers (1987) examined the growth response of 2 to 3 month old pigs in response to DCAB. Feed intakes and growth rates were maximized at a DCAB between 0 and 341 and were reduced at -85. They also noted that blood pH tended to increase as DCAB increased

Haydon and West (1990) examined the effect of DCAB on apparent nutrient digestibilities in pigs fitted with ileal T-cannulas. The DCAB was established by substituting calcium chloride for calcium carbonate and sodium bicarbonate for corn and soybean meal resulting in DCAB of -50, 100, 250, and 400 meq/kg diet dry matter. They noted a linear relationship between DCAB

and apparent ileal digestibility of N, energy, dry matter, and all amino acids except for alanine and methionine. However, nutrient and amino acid digestibilities measured over the total tract were similar. Increasing the DCAB resulted in linear and quadratic effects on daily urinary nitrogen excretion which resulted in a linear improvement in nitrogen retention. Blood pH, total CO2, and HCO3 and base excess concentrations increased linearly with increased DCAB.

From previous work in other species, it is obvious that DCAB has significant physiological effects that alter important production traits. It is also apparent that more work of this type must been done on the horse as the potential impact of DCAB on health and performance is great. This study will investigate the effects of dietary cation-anion balance on mineral balance, blood pH, and urine pH in the anaerobically exercised horse.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Four geldings and four mares of primarily Quarter Horse and Thoroughbred breeding (average weight 463 kg) were randomly assigned treatments within two simultaneous 4 x 4 Latin squares to study the effects of dietary cation-anion balance on mineral metabolism, blood pH and urine pH. The 22 week trial consisted of a 6 week conditioning period and four experimental periods each with 21 days adaptation culminating in 7 days of collection.

Horses were stalled individually and allowed ad libitum access to water. Horses were fed at 10am and 10pm. All horses were immunized and dewormed prior to the initiation of the trial and received standard animal health care throughout the experiment.

Treatments

Diets consisted of a pelleted base concentrate of corn, soybean meal, and cottonseed hulls formulated at the Oklahoma State University Feedmill. The concentrate was fed with bermudagrass hay grown at the OSU Beef Research Center. The concentrate and hay was fed in a 60:40 ratio at levels necessary to maintain constant individual body weights during the 22 week experiment. All

horses were weighed prior to the morning workout one day each week using a standard livestock scale.

Treatments were formed by supplementing the low diet with .78% calcium chloride and .30% ammonium chloride (Table I). The medium-low diet was supplemented with .54% calcium chloride. The high diet was supplemented with .89% potassium citrate and .61% sodium bicarbonate. The medium-high diet was not supplemented and served as the control ration indicative of an industry standard. Diets were calculated to 2.7 Mcal/kg DM and 10.4 % crude protein across treatments (Table II). Further, diets were analyzed and determined to contain equivalent amounts of calcium, phosphorus, magnesium, and sulphur. After supplementation the high diet contained 1.32% potassium and .41% sodium. The medium-low diet contained 1.0% chloride, and the low diet contained 1.38% chloride. These mineral concentrations yielded treatment dietary cation-anion balances of 27, 130, 223, and 354 respectively.

Exercise Regimen

Eight mature horses were conditioned aerobically by galloping 3.2 km/day 6 days each week for 6 weeks on a .8 km oval track located at the Oklahoma State University Equine Center. Workouts consisted of a .4 km warm-up at a long trot and slow gallop. Each horse was conditioned at a pace necessary to maintain a heart rate of 150 beats/min. Following each long slow distance workout (LSD), the horses were warmed down at a slow gallop over .4 km and then walked out for 8 km. As the horses became more fit, the pace was quickened in order to maintain the target heart rate. During the experiment, the horses were subjected to a combined exercise regimen alternating long slow distance with interval training 6 days/week. The LSD workouts consisted of

TABLE I

COMPOSITION OF TREATMENTS,
DRY MATTER BASIS

	Treatment			
Ingredient (%)	L	ML	MH	H
Corn	33.20	33.20	33.20	33.20
Soybean Meal	6.90	6.90	6.90	6.90
Cottonseed Hulls	14.80	15.10	15.00	13.70
Dicalcium Phosphate	.21	.21	.19	.20
Limestone, ground	----	.22	.78	.78
Trace Mineral Salt	.55	.55	.55	.55
Calcium Chloride	.78	.54	----	----
Ammonium Chloride	.30	----	----	----
Potassium Citrate	----	----	----	.89
Sodium Bicarbonate	----	----	----	.61
Molasses, syrup	2.00	2.00	2.00	2.00
Bermudagrass Hay	40.00	40.00	40.00	40.00
Total	100	100	100	100
DCAB, meq((Na+K)-Cl)/kg	+27	+130	+223	+354

TABLE II

TREATMENT ANALYSIS, DRY MATTER BASIS

	Treatment			
Constituent	L	ML	MH	H
DE, Mcal/kg	2.70	2.70	2.70	2.70
Crude Protein, %	10.40	10.40	10.40	10.40
Calcium, %	.50	.53	.52	.54
Phosphorus, %	.28	.29	.28	.28
Magnesium, %	.15	.16	.15	.15
Potassium, %	1.12	1.14	1.13	1.39
Sulfur, %	.11	.12	.11	.13
Sodium, %	.29	.27	.30	.43
Chloride, %	1.38	1.00	.69	.68
DCAB	+27	+130	+223	+354

a 3.2 km gallop at a heart rate of 160 beats/min; gallop times averaged 3 minutes and 10 seconds. The interval training program consisted of a 1.2 km warm up followed by a pair of .4 km sprints each eliciting heart rates of 200-220 beats/min. Between sprints horses were walked until the heart rate recovered to below 110 beats/min. The horses were warmed down for .8 km at a slow gallop and then walked out for another .8 km. When necessary, the horses were rinsed after working. In order to regulate large flucuations in temperature, all exercise occured between the hours of 6am and 7am.

Heart Rate Measurement

During exercise, heart rates were measured using a UNIQ onboard heart rate monitor[a]. Standard EKG skin electrodes were attached at the shoulder and on the stomach near the midline approximately 10 inches behind the girth. In order to improve contact, the hair was clipped with #40 blades, and acetone was used to remove oil from the skin surface before attaching the electrodes. Heart rate was recorded on a 5 second interval throughout the LSD and interval training workouts. The information was then downloaded onto a computer. The data was plotted (heart rate over time) and stored on diskette. These graphs were used to evaluate each workout and individual consistency throughout the trial. Individual jockey weight ranged from 57 to 84 kg with an average of 70 kg across all riders. In order to equilibrate the workload of between individual horses, riders were rotated between horses in sequential order throughout the trial. One rider was picked to perform the standard exercise test for all horses throughout the experiment.

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[a] UNIQ Onboard Heart Rate Monitor (Model 8799), Kempele, Finland.

Blood pH Measurement

Venous Blood Collection

Following the morning workout on the 22nd day of each experimental period, the four geldings were fitted with 18-gauge indwelling jugular catheters. Using #40 blades, the hair was removed from an area covering the jugular approximately 10-12 inches below the throatlatch. This area was then cleaned with betadine solution. With the injection cap exposed, the catheter was then taped in a fixed position. Heparin (3 ml) was injected into the catheter to prevent clotting. To prevent catheter damage while sampling, the horses were tied with access to feed and fresh water. Samples were collected hourly (for 17 hours) beginning at the morning feeding in order to include a 5-hour post-feeding interval with and without the effect of exercise. Just prior to the morning feeding (10 am), 5 ml of blood was drawn from each catheter to remove the heparin saline. Twenty ml of sample was drawn off, and the catheter was reheparinized. This sample was then used to fill a 3 ml and a 7 ml Lithium heparinized blood collection tube. The horse was fed after the time zero sample was drawn. Sampling times were spaced at 5 minute intervals between horses to allow for adequate sampling time. This same procedure was applied to the 4 mares on the 23rd day of each experimental period.

Blood pH Analysis

After sampling, the 3 ml blood tubes were immediately placed (stopper

down) in crushed ice, transported to the lab, and analyzed for pH with a blood gas analyzer[b].

Urine Collection

Geldings

Urine collection harnesses were designed and built in order to measure total urine output from the four geldings. A piece of 102 cm x 102 cm canvas was sewn around 2 cm dow rod on two sides. This piece was placed over a western saddle pad and used to support the collection device. Fence stays were unwound from the closed end until a loop was made. The loop was sized and shaped to fit the sheath of a particular gelding. Automobile tire innertubes were cut in lengths to extend from the sheath, along the stomach midline, and to the sternum. One end was fitted to and sewn around the stay loop. The other end was folded over the stay and clipped to prevent leaking. Four heavy duty rubber bands were put over the stay and around the innertube to support the tube and keep the sample in the middle of the tube. Five adjustable rubber straps, attached to the offside dow rod, extended under the belly, between the stay and the innertube, and attached to the left side dow rod to insure correct positioning of the collection device. To collect a sample, the open end was unclipped from the stay and unfolded so the innertube could be emptied.

Mares

An 46 cm, single bulb, latex catheter was inserted into the mares urethra. The bulb was filled with 60cc saline solution. Ninty-one cm of tubing, attached to

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[b] Instrumentation Laboratories Blood Gas Analyzer (Model 1304), Lexington MA.

the catheter and a collection bag, was braided into the tail. The collection bag was tied into the braid just below the nub of the tail; thus, allowing gravity flow of urine into the bag. Enough slack was given to allow the mare free use of her tail. Therefore, the device was unaffected by defecation.

Beginning on the 22nd day of each experimental period, a total urine collection was taken for 72 hours on the geldings. A 24 hour total urine collection was taken on the mares for 24 hours beginning on the 23rd day of each experimental period. These urine collection devices were drained every four hours. The volume was measured and recorded for each collection. A representative sample (geldings 1%, mares 10%) was composited over time for each horse/period/treatment interval. Another sample (100 ml) was tested for pH using a Fischer Accumet Model 950 pH[c] meter with a standard glass body combination electrode to account for sample temperature. The pH meter was standardized using buffers with pH of 4 and 10 prior to each 4 hour collection measurement. These samples were then acidified to a pH of 2 with hydrochloric acid to prevent bacterial growth. A 20 ml non-acidified sample was taken from each collection for chloride analysis. Individual samples were identified by horse, period, treatment, date, time, and acidified or non-acidified. Composited and individual samples were stored frozen in individual urine cups for subsequent mineral analyses.

Fecal Collection

Chromic oxide was added to the diet as an indigestible marker at the rate of 1.13 kg/ton of concentrate before pelleting. Six rectal fecal grab samples were taken randomly over 72 hours of each collection period such that every 2

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[c] Fisher Accumet pH Meter (Model 950), Fisher Scientific, Pittsburgh, PA.

hours during the post feeding-post exercise interval was represented. Each grab sample was identified by horse, period, treatment, date, and time then stored frozen in freezer safe zip-lock bags.

Laboratory Analyses

Feed and Fecal Mineral

Fecal samples were allowed to thaw overnight at room temperature. Each sample was placed individually in pie pans and dried in a forced air oven at 60°C for 72 hours. These samples were then composited by weight for each horse/period/treatment interval. The feed and composite fecal samples were ground in a Wiley mill through a 1 mm screen and stored in whirl pacs.

Chromium Analysis

Oven-dried 100 ml beakers were weighed then approximately .4 g of fecal material was added, and the air-dried sample weight recorded. All samples were placed in drying ovens at 60°C for 24 hours. The beakers were removed, placed in dessicators, and allowed to cool. Beaker + sample was reweighed to determine oven-dried sample weight. These samples were ashed at 500°C for 4 hours. Six ml of acid mixture (1000 ml DDH, 500 ml H_2SO_4, and 500 ml H_3PO_4) was added to the ashed sample. This mixture was then brought to a boil at a setting of 6 on a hot plate under a hood. Three ml of 4.5% $KBrO_3$ was added, and the mixture was allowed to boil for .5 to 1 minute after SO_3 fumes appeared. The beakers were removed from the hot plate and allowed to cool at room temperature for 10 minutes. Then 20 ml of dilute Bromate was added, and the

mixture was brought to a boil at a setting of 4. After 3-4 minutes, the color changed from clear to milky, and the beaker was removed from heat and allowed to cool. The solution was transferred into 100 ml volumetric flask and filled to volume with distilled, deionized water. The flasks were capped with parafilm and inverted 3 times. Five ml were then transferred to a centrifuge tube and 7.5 ml of 5% NaOH was added. After 15 minutes, the tubes were vortexed and then allowed to settle for 45 minutes. The tubes were then centrifuged at 2000 rpm for 15 minutes. Standards and unknown were then analyzed for chromiun concentration on a spectrophotometer[d] at 400 nm.

Oven-dried 100 ml beakers were weighed then approximately 1.0 g of sample (fecal and feed) was added, and the beaker + air-dried sample weight recorded. The beakers were placed in drying ovens at 60°C for 24 hours. The beakers were then removed, placed in dessicators, and allowed to cool. The beaker + sample was reweighed to determine the oven-dried sample weight. These samples were ashed at 500°C for 4 hours. Once the ashing oven cooled to 120°C the samples were removed, placed in dessicators, and cooled to room temperature. Twenty ml of 20% nitric acid was added. The beaker was placed on a hot plate (setting 2), covered with a watch glass, and the solution was brought to a low boil. After approximately 20 minutes, when the solution turned a dark purple, the beakers were removed and allowed to cool. Each watch glass was rinsed back into the beakers with distilled, deionized water (DDH). The solution was transferred by glass funnel into 100 ml volumetric flask. The beaker and funnel were rinsed into the flask 3 times with DDH. Each flask was

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[d] Gilford Response Series UV-VIS Spectrophotometer, Ciba Corning Diagnostics Corporation.

filled to volume with DDH, covered with parafilm, and inverted 3 times. Both feed and fecal samples were analyzed for sodium, potassium, calcium, phosphorus, magnesium,and sulfur using an Induction Coupled Spectrophotometer[e]. Also, both feed and fecal samples were analyzed for chloride using a Lachat Automated Ion Analyzer[f] .

Urinary Mineral

Calcium, Sodium, Potassium, Chloride
Phosphorus, and Magnesium
Analysis

A ten ml sample was pipetted from each horse/period/treatment composite bottle into 15 ml plastic bottles. One-half ml of HCl was added to keep the solution in suspension. The bottles were inverted three times prior to analysis. Each sample was analyzed for sodium, potassium, calcium, phosphorus, magnesium, and sulfur using an Induction Coupled Spectrophotometer[e] and for chloride using a Lachat Automated Ion Analyzer[f].

Sulfur Analysis

Two ml from each horse/period/treatment composite bottle was pipetted into appropriately labeled 50 ml Erlenmeyer flasks. Then, 5 ml of digestion mixture was added. This mixture consisted of 1.7 g ammonium metavandate in

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[e] Jarrel-Ash Induction Coupled Argon Plasma Spectrophotometer (Model 9000), Allied Analytical Systems, Therno Jarrel-Ash Corporation, Waltham, MA.

[f] QuickChem System IV Automated Ion Analyzer, Lachat Instruments, Milwaukee, WI.

1050 ml concentrated nitric acid (sp.gr. 1.42), 1200 ml perchloric acid (sp. gr. 1.54), and 7.5 g potassium dichromate in 250 ml distilled deionized water. A glass funnel was placed in the nech of each flask before digesting at on an electric hotplate at 80° C for about 15 minutes. After the initial reaction, the digestion was continued at just below boiling (about 190°C) until the perchloric acid is fuming strongly within the flask and an orange precipitate appears. Flask was removed and allowed to cool. The funnel was rinsed back into the flask with deionized water, 25 ml of acid mixture was added, and the digest was diluted to 50 ml with deionized water. The acid mixture consisted of 50 ml glacial acetic acid, 20 ml hydrochloric acid (sp. gr. 1.16), and 20 ml orthophosphoric acid (sp. gr. 1.69) diluted in 1 L of distilled deionized water. The diluted digest mixture was poured into disposable glass cultered tubes for storage until reading. A stock sulfur standard was made by dissolving 5.4341 g of dried potassium sulfate in 1 L deionized water. Working standards were then made by pipetting 0, 1, 2, 3, 4, 5, 6, 7, and 8 ml of stock sulfur solution into 100 ml volumteric flasks. Two ml of a solution containing 15 mg potassium dichromate per ml, 5 ml of perchloric acid, and 50 ml of acid mixture was added and the solution was diluted to volume with distilled deionized water. A solution containing 100 g barium chloride dihydrate and 50 ml of Tween 80 diluted to 1 L was made and allowed to stand overnight before using. Two ml of digested sample and standard solutions were pipetted with 1 ml of the barium chloride-Tween 80 solution into disposable cuvetts prior to being analyzed for sulfur concentration on the spectrophotometer[g] at 623 nm.

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[g] Gilford Response Series UV-VIS Spectrophotometer, Ciba Corning Diagnostics Corporation.

Statistical Analyses

All data were analyzed using a general linear model procedure with horse, period and treatment as main effects. Least squares means were calculated for each variable and Tukey's procedure was used to detect differences between treatment means at ($P < .05$) according to Steele and Torrie (1980).

CHAPTER IV

RESULTS AND DISCUSSION

Blood pH

Mean venous blood pH was lower ($P < .001$) for horses consuming the L diet than for those consuming the MH and H diet at 13 of the 17 measured intervals (Table III and Figure 1). This agrees with the previous work of Baker et al., (1991) noting a decrease in arterial and venous blood pH in horses consuming a low DCAB diet (-50) versus MH and H diets (+150, +250). This also agrees with the depression in blood pH seen in dairy cows fed diets with a DCAB of -100 meq/kg dry matter (Tucker et al., 1988) and with numerous reports in poultry research linking DCAB with the systemic acid-base status of the animal (Cohen, Hurwitz, and Bar, 1972; Cohen and Hurwitz, 1974; Mongin, 1980; Hamilton and Thompson, 1980). Chloride is absorbed from the lumen of the gastrointestinal tract in exchange for the secretion of a bicarbonate ion. This liberates a hydrogen ion from the intermediate carbonic acid (H_2CO_3), resulting in increased systemic acid generation and metabolic acidosis. Hence, blood pH decreases with decreasing DCAB. Peak chloride absorption may have occurred at 1-hour post-feeding across treatments, corresponding to the lowest blood pH values.

TABLE III

THE EFFECT OF DIETARY CATION-ANION BALANCE ON VENOUS BLOOD pH POST-FEEDING IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
Time	L	ML	MH	H	S.E.
10 am[a]	7.391[b]	7.399[b]	7.395[b]	7.398[b]	.005
11 am	7.342[b]	7.368[c]	7.365[c]	7.370[c]	.004
12 pm	7.354[b]	7.378[c]	7.376[c]	7.384[c]	.006
1 pm	7.366[b]	7.380[c]	3.379[c]	7.390[c]	.004
2 pm	7.366[b]	7.381[c]	7.380[c]	7.387[c]	.004
3 pm	7.366[b]	7.381[c]	7.381[c]	7.383[c]	.005
4 pm	7.370[b]	7.385[c]	7.385[c]	7.398[c]	.004
5 pm	7.371[b]	7.376[bc]	7.387[cd]	7.390[d]	.005
6 pm	7.374[b]	7.383[bc]	7.394[cd]	7.395[d]	.004
7 pm	7.375[b]	7.392[c]	7.392[c]	7.400[c]	.004
8 pm	7.382[b]	7.398[c]	7.398[c]	7.395[c]	.004
9 pm	7.383[b]	7.388[b]	7.395[b]	7.391[b]	.004
10 pm[a]	7.396[b]	7.397[b]	7.402[b]	7.398[b]	.003
11 pm	7.348[b]	7.368[b]	7.359[b]	7.356[b]	.007
12 am	7.360[b]	7.378[c]	7.379[c]	7.384[c]	.006
1 am	7.374[b]	7.381[bc]	7.398[d]	7.392[cd]	.005
2 am	7.374[b]	7.377[bc]	7.392[d]	7.387[cd]	.005

[a] Indicates feeding time.
[b,c,d,e] Means in rows with different superscripts differ ($p < .05$).

Urine pH

Variations in urine pH paralleled the blood pH response (Table IV and Figure 2); pH was lowest at 4-hour post-feeding, which corresponded to 2pm and 2am. Horses consuming the H diet had higher ($P < .05$) transient urine pH values than those receiving the other diets. Also, as DCAB increased, mean daily urinary pH increased ($P < .01$) across treatments with values of 6.73, 7.17, 7.38, 7.92. This agrees with Baker and coworkers (1991) who reported a significant decrease in transient urine pH values ranging from 5.40 to 5.86 in sedentary horses fed a calculated DCAB of -50 meq/kg diet DM. The difference in treatment significance between the two trials may be explained by the difference in DCAB values for the H and L treatments. As chloride is filtered from blood and excreted in urine, it is accompanied by either hydrogen, sodium, or potassium. When the hydrogen ion accompanies chloride, urinary pH decreases. Also, there is a metabolic shift of $CO_2 + H_2O$ through the intermediate carbonic acid, ultimately forming $H + HCO_3$ ions. This increases systemic HCO_3 generation.

Dry Matter Digestibility

The effect of DCAB on dry matter digestibility and fecal output is shown in Table V. Fecal dry matter output, expressed in g/d, was calculated by multiplying grams of chromium fed/day times 100 and then divided by the percent chromium in the feces grab sample. Dry matter digestibility was calculated by dividing grams of DM fecal output by the grams of DM intake per day. An increase ($P < .05$) in fecal output and thus a decrease in dry matter digestibility was observed for those horses consuming the L versus H diet. Fecal output increased from 2709 g/d on the H diet to 3134 g/d for those

TABLE IV

THE EFFECT OF DIETARY CATION-ANION BALANCE ON URINE pH POST-FEEDING IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
Time	L	ML	MH	H	S.E.
10AM[a]	6.80[b]	7.18[c]	7.43[c]	7.99[d]	.11
2PM	6.84[b]	7.04[bc]	7.23[c]	7.77[d]	.10
6PM	6.87[b]	7.20[bc]	7.52[c]	8.01[d]	.12
10PM[a]	6.71[b]	7.25[c]	7.53[c]	8.15[d]	.10
2AM	6.59[b]	7.11[c]	7.20[c]	7.74[d]	.11
6AM	6.64[b]	7.29[c]	7.45[c]	7.96[d]	.12
Average	6.73[e]	7.17[f]	7.38[g]	7.92[h]	

[a] Indicates feeding time

[b,c,d] Means in rows with different superscripts differ ($P < .05$).

[e,f,g,h] Means in rows with different superscripts differ ($P < .01$).

TABLE V

THE EFFECT OF DIETARY CATION-ANION BALANCE ON DRY MATTER DIGESTIBILITY IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
DM Digestibility %	61.63[a]	65.41[ab]	63.54[ab]	66.82[b]	1.05
Fecal Output g/d	3134[a]	2825[ab]	2978[ab]	2709[b]	85.99

[a,b] Means in rows with different superscripts differ ($P < .05$).

horses consuming the L diet. Accordingly, dry matter digestibility decreased from 66.82% to 61.63%. The ML and MH diet values were intermediate (65.41% and 63.54% respectively) and were not statistically different from the H or L diets. This disagrees with Nelson and coworkers (1981) who reported decreases in dry matter and amino acid digestion in 30-d-old chicks fed diets that contained the greatest cation-anion ratio. However, they manipulated cation-anion ratio by supplementing with calcium or phosphorus. The present findings do agree with Yen et al. (1981) who observed that the addition of 4% calcium chloride to the diet resulted in reduced feed intake, weight gain, and feed efficiency in crossbred barrows and with Haydon and West (1990) who reported a linear relationship between DCAB and apparent ileal digestibility of Nitrogen, energy, dry matter, and all amino acids, except for alanine and methionine in diets with DCAB of -50 to 400 meq/kg od diet dry matter. However, nutrient and amino acid digestibilities measured over the total tract were similar. The previous results might be explained by Riley and Austic (1983) who reported the pH of the crop was depressed by dietary chloride. However, neither chloride nor potassium had an effect on the pH of the proventriculus, duodenum, or the middle and distal protions of the small intestine. Most digestive enzymes have optimal activity at a pH ranging from 6.5 to 7.5. Therefore, if decreasing the DCAB increases the acidity of the digestive tract, the activity of many pH sensitive digestive enzymes may be decreased.

Sodium Balance

The high diet was supplemented with .89% potassium citrate and .61% sodium bicarbonate, increasing sodium intake to 35.38 g/d as compared to 24.02, 22.36, and 24.33 g/d for the L, ML, and MH diets respectively. This effect

of DCAB on sodium balance is shown in Table VI and Figure 3. No differences in fecal sodium excretion were detected between the four treatments. However, urinary sodium excretion paralleled intake. Sodium excretion was similar for the L, ML, and MH diets (8.57, 8.61,and 5.94 g/d respectively). The increased daily sodium intake for those horses consuming the high diet resulted in a significant increase in daily urinary sodium excretion to 14.03 g/d. This increase in daily urinary excretion did not offset the increased intake as those horses on the high diet retained more sodium (8.86 g/d) as compared to the L (3.47 g/d) and ML (2.08 g/d). The MH diet (5.36 g/d) was not different from the other treatments. These findings agree with those of Schyrver and coworkers (1987) who demonstrated that urinary excretion was the primary pathway for sodium loss in sedentary horses consuming 1, 3, and 5% sodium chloride. He noted that sodium intake was directly related to urinary sodium excretion but had no effect on fecal excretion, intestinal absorption, or retention of sodium. The difference in sodium retention might be explained by the absolute amount and/or form of the sodium supplement used in the two trials.

Young and coworkers (1989) reported sweat sodium losses of 42.1 mg/kg BW in heavily exercised miniature horses consuming 25.4 mg/kg BW. Their findings agree with Meyer (1987) who suggested that most diets contain inadequate sodium concentrations to meet the needs of the exercising horse. He estimated the sweat sodium loss to range from 8.25 to 82.5g depending on the level of exercise. Although all treatments exhibited positive sodium balances, we may assume that these horses were marginal, if not in negative sodium balance, due to the large amount of sodium potentially lost in sweat.

From the present study, it can be suggested that DCAB increases the sodium balance of anaerobically exercised horses and that this increase may help offset the large amount of sodium lost in sweat. The NRC (1989) lists the

TABLE VI

THE EFFECT OF DIETARY CATION-ANION BALANCE ON SODIUM BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	24.02	22.36	24.33	35.38	
Urine g/d	8.57[a]	8.61[a]	5.94[a]	14.03[b]	.96
Fecal g/d	11.97[a]	11.67[a]	13.06[a]	12.48[a]	.99
Balance g/d	3.47[a]	2.08[a]	5.36[ab]	8.86[b]	1.18

[a,b] Means in rows with different superscripts differ ($P < .05$).

sodium requirement of working horses at .30% DM. From this study and estimations of sweat sodium losses, this value may be inadequate. Further research on the sodium balance of the exercising horse is needed to accurately quantify the sodium requirement.

Potassium Balance

Potassium, one of the cations used to manipulate the DCAB, was supplemented as potassium citrate at .89% along with .61% sodium bicarbonate in the H diet (Table I). Thus, daily potassium intake increased to 113.78g as compared to L (91.85g), ML (93.75g), and MH (92.37g). This effect of DCAB on potassium balance is shown in Table VII and Figure 4. Those horses consuming the L diet had higher ($P < .05$) fecal excretion of potassium (22.29 g/d) as compared to the other treatments (ML=17.52, MH=17.35, and H=17.46 g/d). Daily urinary excretion of potassium paralleled intake. The increase in intake to 113.78 g/day in the H diet resulted in a significant increase ($P < .05$) in daily urinary potassium excretion (73.95 g/d). Potassium excretion was similar for the L (50.74), ML (49.38), and MH (50.33 g/d) diets. The increase in urinary excretion in the H diet and the decrease in intestinal absorption in the L diet did not produce significant differences in potassium balance (L=18.82, ML=26.46, MH=24.69, and H=22.38 g/d); however, there was a trend for balance to decrease on the L diet.

The NRC (1989) lists the potassium requirement at 1.52 times Mcal of DE intake/d. Young and coworkers (1989) reported sweat potassium losses of 138.1 mg/kg BW in heavily exercised miniature horses consuming 220.2 mg/kg BW. These horses were in a slight negative potassium balance. However, Meyer (1987) suggested that the potassium requirement was adequate in

TABLE VII

THE EFFECT OF DIETARY CATION-ANION BALANCE ON POTASSIUM BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	91.85	93.37	92.37	113 78	
Urine g/d	50.74[a]	49.38[a]	50.33[a]	73.95[b]	4.31
Fecal g/d	22.29[a]	17.52[b]	17.35[b]	17.46[b]	1.06
Balance g/d	18.82[a]	26.46[a]	24.69[a]	22.38[a]	4.53

[a,b] Means in rows with different superscripts differ ($P < .05$).

meeting the demands of the exercised horse. The horses in this trial consumed 24.55 Mcal/d thus requiring 37.32 g/d of potassium. As in most horse rations, the potassium intake exceeded the requirement. Thus, considering sweat loss, we may suggest that if fed at the recommended level these horses could have been in negative potassium balance independent of DCAB.

Chloride Balance

Chloride was the only anion used to manipulate the DCAB in this experiment. The ML diet was supplemented with .54% calcium chloride and the L diet was supplemented with .78% calcium chloride along with .30% ammonium chloride. Thus, daily intake of chloride was increased to 81.36 g/d for the ML diet and to 112.40 g/d for horses consuming the L diet. This compares with 56.62 g/d and 55.17 g/d for the MH and H diets. The effect of altering the DCAB on chloride balance in shown in Table VIII and Figure 5. No difference was detected in fecal chloride excretion across treatments. However, decreasing the DCAB resulted in increased ($P < .05$) urinary chloride excretion in the L (67.17 g/d) and ML (56.14 g/d) as compared to the MH (33.05 g/d) and H (35.39 g/d) diets. Apparently, the increase in urinary chloride excretion and the loss of chloride in the sweat was sufficient to offset the increased chloride intake in the ML diet as daily chloride balance was similar for the ML (17.00g), MH (17.07g), and H (12.04g). However, these chloride elimination pathways were not adequate in removing the excess chloride in the L diet as only the L diet proved to retain more chloride (37.65 g/d).

These results agree with other data demonstrating increased urinary chloride excretion in horses consuming diets with a lower DCAB (Topliff et al., 1989). However, this study disagrees with Schryver and coworkers (1987) who

TABLE VIII

THE EFFECT OF DIETARY CATION-ANION BALANCE ON CHLORIDE BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	112.40	81.36	56.62	55.17	
Urine g/d	67.17[a]	56.14[a]	33.05[b]	35.39[b]	4.13
Fecal g/d	7.58[a]	8.22[a]	6.49[a]	7.74[a]	.86
Balance g/d	37.65[a]	17.00[b]	17.07[b]	12.04[b]	4.13

[a,b] Means in rows with different superscripts differ ($P < .05$).

reported dietary chloride to be completely absorbed in sedentary horses consuming diets with 1, 3, and 5% sodium chloride and that urinary excretion was the sole pathway for chloride elimination.

Meyer (1987) suggested that the increase in chloride requirement of the exercising horse was met by the obligatory increase in dry matter intake necessary to meet energy demands. However, Young and coworkers (1989) reported exercised miniature horses with a daily chloride intake of 40.9 mg/kg BW to have sweat chloride losses of 90.3 mg/kg BW. They suggested that the increase in chloride intake accompanying the increase in dry matter intake associated with exercise was not sufficient to balance the large amount of chloride lost in sweat. These differences may be explained by the variance in workload and climatic factors.

The NRC (1989) suggests that chloride requirements are presumed to be met when the sodium requirement is met by supplementing the diet with sodium chloride. However, Young and coworkers (1989) fed approximately 1.5 times more chloride than sodium to exercising miniature horses and still experienced a chloride deficiency. In the present study, a chloride to sodium ratio of 4.76 resulted in a chloride balance of 37.65 g/d not including the chloride lost in sweat. Whereas, ratios of 3.7 (ML), 2.3 (MH), and 1.58 (H) produced similar chloride balances of 17.00, 17.07, and 12.04 g/d respectively.

From this study, we may suggest that diets with low DCAB do increase the chloride balance in the anaerobically exercised horse depending on the level of chloride lost in sweat. Furthermore, this increased chloride balance has marked effects on blood and urine pH along with calcium, phosphorus, magnesium, and sodium balance in the anaerobically exercised horse.

Magnesium Balance

Magnesium has been implicated as playing a minor role in the DCAB equation in dairy cattle. Therefore, the magnesium concentration was purposely held constant across treatments in order to evaluate sodium, potassium, and chloride as the primary contributers to the DCAB equation. The effects of DCAB on magnesium balance are shown in Table IX and Figure 6. Intake of magnesium varied slightly across treatments ranging from 12.41 to 12.69 g/d. The L diet resulted in an increase ($P < .05$) in fecal magnesium excretion to 7.59 g/d. The ML, MH, and H diets were similar with fecal excretions of 6.34, 6.45, 6.47 g/d. It appears that urinary excretion of magnesium was not affected by DCAB. Due to the increased fecal excretion, magnesium balance was lower ($P < .05$) for those horses consuming the L diet (.94g/d) versus the other treatments (ML=2.65, MH=2.28, H=2.31 g/d).

The NRC (1989) suggests the magnesium requirement is .46 times Mcal DE intake/day. Therefore, the horses in this trial required 11.29 g/d. Accordingly, all diets should have been sufficient in meeting the requirement. However, we may suggest that those horses consuming the L diet could be in marginal if not negative magnesium balance.

Sulfur Balance

Sulfur is an ion sometimes used in the equation to express DCAB. Therefore, by design, the intake of sulfur was held similar across treatments ranging from 9.20 g/d for the L diet to 10.39 g/d for those horses consuming the H diet. The effects of DCAB on sulfur balance is shown in Table X and Figure 7. DCAB did not appear to affect sulfur balance as values for urinary and fecal sulfur excretion were similar across treatments. Urinary excretion ranged

TABLE IX

THE EFFECT OF DIETARY CATION-ANION BALANCE ON MAGNESIUM BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	12.41	12.69	12.51	12.48	
Urine g/d	3.88[a]	3.70[a]	3.78[a]	3.70[a]	.31
Fecal g/d	7.59[a]	6.34[b]	6.45[b]	6.47[b]	.24
Balance g/d	.94[a]	2.65[b]	2.28[ab]	2.31[ab]	.34

[a,b] Means in rows with different superscripts differ ($P < .05$).

TABLE X

THE EFFECT OF DIETARY CATION-ANION BALANCE ON SULFUR BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	9.20[a]	9.49[a]	9.26[a]	10.39[a]	
Urine g/d	7.91[a]	9.03[a]	8.34[a]	8 73[a]	1.93
Fecal g/d	2.54[a]	2.35[a]	2.31[a]	2 23[a]	.14
Balance g/d	-1.25[a]	-1.89[a]	-1.39[a]	-0.56[a]	1.91

[a,b] Means in rows with different superscripts differ ($P < .05$).

from 7.91 to 9.03 g/d while fecal excretion varied from 2.23 to 2.54 g/d. Although no significant differences were detected, the sulfur balance was negative across treatments (L = ⁻1.25, ML = ⁻1.89, MH = ⁻1.39, and H = ⁻.56).

The NRC (1989), based on the work of Jarrige and Martin-Rosset (1981), suggests the sulfur requirement of the exercising horse is .15%, a minimum value provided by most high quality protein sources. In the present study, the sulfur content of the feedstuffs was overestimated resulting in sulfur concentrations slightly below the requirement (L=.11, ML=.12, MH=.11, and H=.13%). Therefore, these horses consumed about 2 to 3 g/d below the suggested 12.25 g/d. This could be the likely explanation for the negative sulfur balances. The NRC (1989) states the apparent protein digestibility for diets with a concentrate-to-hay ratio above 1:1 to be 70 to 75% depending on the source and need of the animal. In this study, the apparent absorption efficiency of sulfur averaged 75.31% across treatments.

Recent research conducted by Tucker et al., (1991) demonstrated that dietary chloride and sulfur had similar effects on the acid-base status of dairy cows. This is in agreement with Oetzel (1991) who analyzed the previous research conducted on acid-base balance in dairy cattle and determined that sulfur is the primary ion affecting acid-base balance. Therefore, they suggested that sulfur be included along with chloride in the DCAB equation for lactating dairy cows.

Additional research investigating the role of sulfur in the acid-base physiology of anaerobically exercised horses is necessary to substantiate the inclusion of sulfur in the DCAB equation.

Phosphorus Balance

The effect of DCAB on phosphorus balance is shown in Table XI and Figure 8. The intake of phosphorus was held constant across treatments (L=22.77, ML=23.95, MH=22.86, and H=22.94). DCAB affected phosphorus in the same manner as magnesium, decreasing ($p < .05$) intestinal absorption in horses consuming the L diet. These horses had higher fecal phosphorus excretion (21.68 g/d) as compared to the other treatments (ML=17.74, MH=17.18, H=17.20). The DCAB did not appear to affect renal excretion of phosphorus; excretion ranged from .06 - .07 g/day across treatments. The low excretion values may be due to renal retention of phosphorus under situations of dietary and/or exercise induced urinary calcium loss and the excretion of phosphate in the feces. Also, there is some indication that phosphorus may be changed from the organic to inorganic form even in the frozen state. Additional phosphorus analysis using a Cobas Mira Automated Ion Analyzer was done to test the original measurements. These test paralleled the previous urinary phosphorus analysis producing values slightly less than before across all tested samples, possibly due to the conversion from the organic to inorganic form.

The increased fecal excretion of phosphorus in those horses consuming the L diet resulted in a decrease ($P < .05$) in phosphorus balance (1.03 g/d) as compared to 6.16 g/d (ML), 5.62 g/d (MH), and 5.68 g/d (H). This same effect seen in potassium and magnesium may be associated with the decrease in dry matter digestibility as these are primarily intracellular and structural elements.

The NRC (1989) suggests the phosphorus requirement is .87 times Mcal DE/d. Therefore, the horses in the present study required 21.36 g/d. Considering sweat phosphorus losses are minimal, each treatment supplied

TABLE XI

THE EFFECT OF DIETARY CATION-ANION BALANCE ON PHOSPHORUS BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	22.77	23.95	22.86	22.94	
Urine g/d	.07[a]	.06[a]	.06[a]	.06[a]	.01
Fecal g/d	21.6[a]	17.74[b]	17.18[b]	17.20[b]	.42
Balance g/d	1.0[a]	6.16[b]	5.62[b]	5.68[b]	.42

[a,b] Means in rows with different superscripts differ ($P < .05$).

adequate phosphorus. However, from these data we may suggest that a low DCAB decreases intestinal absorption and retention of phosphorus to near marginal levels in the anaerobically exercised horse.

Calcium Balance

The effect of DCAB on calcium balance is shown in Table XII and Figure 9. The calcium concentration across treatments was formulated to be constant across treatments in order to more accurately quantify the effects of DCAB on calcium balance. However, due to variation in feedstuff composition calcium intake across treatments was 40.82 g/d (L), 42.98 g/d (ML), 42.35 g/d (MH), and 44.22 g/d (H). The effects of DCAB on intestinal absorption are not consistant with the other minerals. Fecal calcium excretion was higher ($P < .05$) for those horses consuming the H diet (21.01 g/d) versus the ML diet (15.66 g/d). This effect is basically opposite those of the other minerals, but may be explained by the calcium homeostatic control mechanisms. These horses also had decreased ($P < .05$) urinary calcium excretion (10.33 g/d) versus those horses consuming the L diet (20.11 g/d). The ML and MH diets were intermediate and not different from either the H or L diet.

These findings agree with published data demonstrating increased urinary calcium excretion in horses (Topliff et al., 1989), rabbits (Thacker, 1959), rats (Barzel and Jowsey, 1989; Newell and Beauchene, 1975; Cole and Zlotkin, 1983; Petito and Evans, 1984; Emerick, 1984; Goulding and Campbell, 1984), and humans (Walser and Browder, 1959; Lemann and Relman, 1959; Kleeman et al., 1964; Adams et al., 1979; Schuett et al., 1980) consuming diets of lower DCAB.

TABLE XII

THE EFFECT OF DIETARY CATION-ANION BALANCE ON CALCIUM BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

	Treatment				
	L	ML	MH	H	S.E.
Intake g/d	40.82	42.98	42.35	44.22	
Urine g/d	20.11[a]	15.71[ab]	12.16[ab]	10.33[b]	2.12
Fecal g/d	17.45[ab]	15.66[a]	19.53[ab]	21.01[b]	.97
Balance g/d	3.26[a]	11.61[ab]	10.66[ab]	12.88[b]	2.29

[a,b] Means in rows with different superscripts differ ($P < .05$).

Parathyroid hormone has been shown to have a more dramatic effect on renal production of 1,25-dihydroxyvitamin D in dairy cows fed highly anionic diets thus increasing intestinal calcium absorption (Goff et al., 1991). Also, osteoclastic bone resorption was more responsive to parathyroid hormone as plasma hydroxyproline concentration was higher in those cows fed the low DCAB diet. The parathyroid hormone activity might be due to the decrease in the pH of the blood associated with diets of lower DCAB.

These changes in fecal and urinary calcium metabolism resulted in an increase ($P < .05$) in calcium balance of those horses consuming the H diet (12.88 g/d) as compared to those on the L diet (3.26 g/d). The ML and MH diets were again intermediate and not different from either the H or L diet.

The NRC (1989) suggests the calcium requirement to be 1.22 times the Mcal of DE intake/day. These horses would therefore require 29.95 g/d of calcium. We purposely exceeded the calcium level in the diet as not to predispose these horses to a calcium defiency; therefore, each treatment has a 10 to 12 g cushion in calcium requirement. Because of the tight control of calcium homeostatic mechanisms on intestinal absorption and renal reabsorption, it is not feasible to say that all horses would be in negative or marginal calcium balance if we had fed calcium levels more near the requirement. However, we may suggest that as DCAB decreases calcium balance also decreases, predisposing those animals to negative calcium balance. When prolonged, this condition could lead to an osteoporotic weakening of the skeletal system as seen in poultry (Leach and Neshium, 1965; Leach and Neshium, 1972; Sauveur and Mongin, 1978; Edwards, 1984; Hallet et al.m 1987; Hamilton and Thompson, 1980; Hurwitz et al., 1973; and Mongin, 1981).

Summary and Conclusions

These results further indicate the direct correlation between dietary cation-anion balance and the acid-base status of the animal. Furthermore, this correlation is positive noted by the decreasing pH of blood and urine in animals fed decreasing DCAB diets.

These results also provide additional information concerning mineral balance and the implications of DCAB in the exercised horse. It is difficult to make inferences toward the mineral requirement of the heavily exercised horse without the knowledge of sweat rate and composition. However, these results suggest that the NRC (1989) recommendations for, magnesium, sodium, and possibly potassium may not meet the demands of the anaerobically exercised horse. We may also conclude that exercising horses consuming highly anionic diets experience decreases in calcium, magnesium, phosphorus, and sodium balance and that these could easily be negative depending on the level of intake.

From the previous discussion, it should be clear that both the absolute levels and the ratios of electrolytes in horse rations should be precisely controlled. However, it is a common industry practice to use feed additives, top dress with various mineral mixtures, and to use compounds such as Lasix that alter the mineral balance of the animal. There are many known factors that affect the mineral balance of the exercising horse that need to be further quantified including temperature and humidity, degree and intensity of workload, water intake, and the possibility of other acidogenic or alkalogenic agents.

Also, further research is needed to determine the effects of DCAB in the young rapidly growing horse which would be particularly susceptible to alterations in mineral balance that might affect bone formation.

LITERATURE CITED

Adams, N.D., R.W. Gray and J. Lemann, Jr. 1979. The calciuria of increased fixed acid production in humans: evidenced against a role for parathyroid hormone and 1,25-(OH)2 - vitamin D. Calcif. Tiss. Int. 28:233-238.

Anderson, R.S., 1967. Acid-base changes in excreta of the laying hen. Vet. Rec. 80:315-315.

Austic, R.E. 1984. Excess dietary chloride depresses eggshell quality. Poutlry Sci. 63:1773-1777.

Austic, R.E. 1988. Dietary mineral balance: its relationship to acid-base homeostasis in poultry. Proc. California Anim Nutr. Conf. Fresno, Ca.

Barzel, U.S. and J. Jowsey. 1969. The effects of chronic acid and alkali administration on bone turnover in adult rats. Clin. Sci. 36:517-524.

Beighle, D.E., W.B. Tucker, and R.W. Hemken. 1990. Interactions of dietary cation-anion balance and phosphorus effects on blood, bone and faecal phosphorus concentrations in dairy calves. Tydskr. S. Afr. Vet. Ver. 61:(1):5-8.

Bergstrom J., G. Guarnieri, and E. Hultman. 1971. Carbohydrate metabolism and electrolye changes in human muscle tissue during heavy work. J. Appl. Physiol. 30:122.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce the incidence of milk fever. J. Dairy Sci. 67:2939.

Chan, J.C.M. 1974. The influence of dietary intake on endogenous acid production. Nutr. Metab. 16:1.

Cohen, I. and S. Hurwitz. 1974. The response of blood ionic constituents and acid-base balance to dietary sodium, potassium and chloride in laying fowls. Poultry Sci. 53:378-383.

Cohen, I., S. Hurwitz and A. Bar. 1972. Acid-base balance and sodium-to-chloride ratio in diets of laying hens. J. Nutr. 102:1-8.

Cole, D.E.C. and S.H. Zlotkin. 1983. Increased sulfate as an etiological factor in hypercalciuria associated with total parenteral nutrition. Am. J. Clin. Nutr. 37:108-113.

Coppock, C.E. 1986. Mineral utilization by the lactating cow - chlorine. J. Dairy Sci. 69:595.

Dishington, I.W. 1975. Prevention of milk fever (hypocalcemic paresis puerperalis) by dietary salt supplementation. Acta Vet. Scand. 16:503.

Drepper, K., J.O. Gutte, H. Meyer and F. J. Schwarz. 1982. Energie- und Nahrstoffbedarf landwirtrschaftlicher Nutztiere. Nr. 2 empfehlungen zur energie- und nahrstoffversorgung der Pferde. Frankfurt am Main, Germany:DLG Verlag.

Edwards, H.M., Jr. 1984. Studies on the etiology of tibial dyschondroplasia in chickens. J. Nutr. 114:1001-1013.

Emerick, R.J. 1984. Chloride and phosphate as impediments to silica urinary calculi in rats fed tetraethylorthosilicate. J. Nutr. 114:733-738.

Ender, F., R.J. Dishington and A. Hlegebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. Z. Tierphysiol. Tierernahr., Futtermittelkd. 28:233.

Escobosa, A., C.E. Coppock, L.D. Rowe, Jr., W.L. Jenkins, and C.E. Gates. 1984. Effects of dietary sodium bicarbonate and calcium chloride on physiological responses of lactating dairy cows in hot weather. J. Dairy Sci. 67:574.

Frank, F.R. and R.E. Beger. 1965. The effect of carbon dioxide inhalation and sodium bicarbonate ingestion on egg shell deposition. Poultry Sci. 44:1604.

Gilligan, D.R., M.D. Altschule, and E.M. Katershy. 1943. Physiological intravascular hemolysis of exercise. Hemoglobinuria and hemoglobinemia following cross-country runs. J. Clin. Invest. 22:859.

Goff J.P., R.L. Horst, F.J. Mueller, J.K. Miller, G.A. Kiess, and H.H. Dowlen. 1991 Addition of chloride to a prepartal diet high in cations increases 1,25 dihydroxyvitamin D response to hypocalcemia preventing milk fever. J Dairy Sci 74:3863-3871.

Golz, D.I. and T.D. Crenshaw. 1990. Interrelationships of dietary sodium, potassium and chloride on growth in young swine. J. Anim. Sci. 68:2736.

Goulding, A. and .R. Campbell. 1984. Effect of oral loads of sodium chloride on bone composition in growing rats consuming ample dietary calcium. Mineral Electrolyte Metab. 10:58-62.

Hall, K.N. and N.V. Helbacka. 1959. Improving albumin quality. Poultry Sci. 38:111-114.

Halley, J.T., T.S. Nelson, L.K. Kirby and Z.B. Johnson. 1987. Effect of altering dietary mineral balance on growth, leg abnormalities, and blood base excess in broiler chicks. Poultry Sci. 66:1684-1692.

Hamilton, R.M.G. and B.K. Thompson. 1980. Effects of sodium plus potassium to chloride ratio in practical-type diets on blood gas levels in three strains of white leghorn hens and the relationship between acid-base balance and eggshell strength. Poultry Sci. 59:1294-1303.

Haydon, K.D. and J.W. West. 1990. Effect of dietary electrolyte balance on nutrient digestibility determined at the end of the small intestine and over the total digestive tract in growing pigs. J. Anim. Sci. 68:3687-3693.

Hintz, H.F. and H.F. Schryver. 1972. Magnesium metabolism in the horse. J. Anim. Sci. 35:755.

Hintz, H.F. and H.F. Schryver. 1976. Potassium metabolism in ponies. J. Anim. Sci. 42:637.

Howes, J.R. 1967. Acid-base relationships and calcium deposition in the egg shell. 22nd Distillers Feed Conference. p.32.

Hunt, J.R. and J.R. Aitken. 1962. The effect of ammonium and chloride ions in the diet of hens on egg shell quality. Poultry Sci. 41:434-438.

Hurwitz, D., I. Cohen, A. Bar and S. Bornstein. 1973. Sodium and chloride requirements of the chick: relationship to acid-base balance. Poultry Sci. 52:903.

Jarrige, R., and W. Martin-Rosset. 1981. Le cheval: Reproduction, selection, alimentation, exploitation. XIII Journees du Grenier Theix. Paris: Institut National de la Recherche Agronomique.

Kelso, T. B., D.R. Hodgson, E.H. Witt, W.M. Bayly, B.D. Grant and P.D. Gollnick. 1986. Bicarbonate administration and muscle metabolism during high-intensity exercise. Proc. Second Inter. Conf. Equine Exercise Physiology. San Deigo, CA. p.438.

Kerr, M.G. and D.H. Snow. 1983. Composition of sweat in horses during prolonged epinephrine (adrenaline) infusion, heat exposure and exercise. Am. J. Vet. Res. 44:1571.

Kjellmer, I. 1965. The potassium ions as a vasodilator during muscular exercise. Acta Physiol. Scand. 63:460.

Kleeman, C.R., J. Bohannan, D. Bernstein, S. Ling, and M.H. Maxwell. 1964. Effect of variations in sodium intake on calcium excretion in normal humans. Proc. Soc. Exptl. Biol. Med. 115:29-32.

Lawrence, L.M., K. Kline, P. Miller-Graber, a. Siegel, E. Kurcz, M. Fisher and K. Bump. 1990. Effect of sodium bicarbonate on racing standardbreds. J. Anim. Sci. 68:673.

Lawrence, L.M., P.A. Miller, P.J. Bechtel, B. Kane, E. Kurcz and J. Smith. 1986. The effect of sodium bicarbonate ingestion on blood parameters in exercising horses. Proc. Second Inter. Conf. Equine Exercise Physio. San Diego, CA. p. 448.

Leach, R.M., Jr. and M.C. Neshiem. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. Nutr. 86:236-244.

Leach, R.M., Jr. and M.C. Neshiem. 1972. Further studies on tibial dyschondroplasia (cartilage abnormality) in young chicks. J. Nutr. 90: 310.314.

Lemann, J., Jr. and A.S. Relman. 1959. The relationship of sulphur metabolism to acid-base balance and eletrolyte excretion: the effects of DL-Methionine in normal man. J. Clin. Invest. 38:2215-2223.

Lomba, F., G. Chauvaux, E. Teller, L. Lengels, and V. Bienfet. 1978. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. Br. J. Nutr. 39:425.

McKenzie, R.A., B.J. Blaney, and R.J. W. Gartner. 1981. The effect of dietary oxalate on calcium, phosphorus and magnesium balances in the horse. J. Agr. Sci. Camb. 97:69.

Melliere, A.L. and R.M. Forbes. 1966. Effect of altering the dietary cation-anion ratio on food consumption and growth of young chicks. J. Nutr. 90:310-314.

Meyer, H. 1979. Magnesium stoffwechsel und magnesium bedarf des pferdes obers. Z. Tierernahrg Band. 7:75.

Meyer, H. 1987. Nutrition of the equine athlete. Equine Exercise Physiology II, ICEEP Publications. Davis, Calif. 650-666.

Meyer, H., C. Gurer and A. Lindner. 1985. Influence of low K ıntake on K metabolism, sweat production, and sweat composition in ponies. Proc. Ninth Equine Nutr. and Phssio. Symp. E. Lansing, MI p. 130.

Meyer, H., A. Lindner, M. Schmidt and H.M. Teleb. 1983. Investigations of sodium defiency in horses. Proc. Eight Equine Nutr. Physio. Symp. Lexington, Ky.

Milne, D.W. 1974. Blood gases, acid-base balance and electrolyte and enzyme changes in exercising horses. Jl. S. Afr. Ass. 45(4):345-354.

Mongin, P. 1968. Role of acid-base balance in the physiology of egg shell formation. World's Poultry Sci. J. 24:200

Mongin, P. 1978. Acid-base balance during eggshell formation. Respiratory Function in Birds. Adult and Embryonic. p.247-259.

Mongin, P. 1980. Electrolytes in nutrition: Review of basic principles and practical application in poultry and swine. Proc. Third Annu. Int. Miner. Confer., Orlando, Fl. p. 1-15.

Mongin, P. 1981. Recent advances in dietary cation-anion balance: applications in poultry. Proc. Nutr. Soc. 40:285.

Morgen, A. and C. Beger. 1915. Uber den schaddechen, auf eine sauerevergiftung zur uckzufuhren den einfluss winer ausschliess lichen haferfutterung. Z. Physiol. Shem., 94:324.

Mottershead, B.E. Estimation of sulphur in biological materials using the technicon autoanalyser. Laboratory Practice. 20(6):483-484&491.

Nelson, T.S., Kirby, L.K., and Johnson, Z.B. 1981. The effect of altering the cation-anion content with calcium and phosphorus on the digestion of dry matter and amino acids and on energy utilization. Poultry Sci. 60:786.789.

Neshium, M.C., R.M. Leach, Jr., T.R. Zeigler, and J.A. Serafin. 1964. Interrelationships between dietary levels of sodium, chlorine and potassium. J. Nutr. 84:361.

Newell, G.K. and R.E. Beauchene. 1975. Effects of dietary calcium level, acid stress, and age on renal, serum, and bone reponses in rats. J. Nutr. 105:1039-1047.

NRC. 1966. Committee on Animal Nutrition. Nutrient requirements of horses. National Acedemy of Sciences, National Research Council, Washington, DC.

NRC: 1989. Nutrient Requirements of Domestic Animals. Nutrient requirements of horses. 5th Revised Ed. National Academy of Sciences, National Research Council. Waxhington, DC.

Oetzel, G.R., J.D. Olson, C.R. Curtis, and M.J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302.

Patience, J.F., R.E. Austic and R.D. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64:457-466.

Petito, S.L. and J.L. Evans. 1984. Calcium status of the growing rat as affected by diet acidity from ammonium chloride, phosphate and protein. J. Nutr. 114:1049-1059.

Pugh, L.G., J.L. Corbett, and R.H. Johnson. 1967. Rectal temperature, weight loss and sweat rates in marathon running. J. Appl. Physiol. 23:347.

Riley, W.M., Jr. and R.E. Austic. 1983. Influence of dietary electrolytes on digestive tract pH and acid-base status of chicks. Poultry Sci. 63:2247-2251.

Rose L.I., D.R. Caroll, S.L. Lowes, E.W. Peterson, and K.H. Cooper. 1970. Serum electrolyte changes after marathon running. J. Appl. Physiol. 29:449.

SAS Institute Inc. 1985. SAs User's Guide: Statistics, Version 5 Edition. Cary NC: SAS Institute Inc.

Sauveur, B. and P. Mongin. 1971. Etude comparative du fluide uterin et de l'albumen de l'oeuf in utero chez la poule. Ann. Biol. Anim. Biochem. Biophys. 11:213-224.

Sauveur, B. and P Mongin. 1978. Tibial dyschondroplasia, a cartilage abnormality in poultry. Ann. Biol. Anim. bioch. Biophys. 18:87-98.

Schryver, H.F., P.H. Craig and H.F. Hintz. 1970. Calcium metabolism in ponies fed varying levels of calcium. J. Nutr. 100:955.

Schryver, H.F., H.F. Hintz, and P.H Craig. 1971a. Calcium metabolism in ponies fed a high phosphorus diet. J. Nutr. 101:259.

Schryver, H.F., H.F. Hitnz, and P.H. Craig. 1971b. Phosphorus metabolism in ponies fed varying levels of phosphorus. J. Nutr. 101:1257.

Schryver, H.F., H.F. Hintz and J.E. Lowe. 1978. Calcium metabolism, body composition and sweat losses of exercised horses. Am. J. Vet. Res. 39:245.

Schryver, H.F., M.T. Parker, P.D. Daniluk, K.I. Pagan, J. Williams, L.V. Soderholm, and H.F. Hintz. 1987. Salt consumption and the effect of salt on mineral metabolism in horses. Cornell Vet. 77:122.

Schuettt, S.A., M.B. Zemel, and H.M. Linkswiler. 1980. Studies on the mechanism of protein-induced hypercalciuria in older men and women. J. Nutr. 110:305-315.

Thacker, E.J. 1959. Effect of a physiological cation-anion imbalance on the growth and mineral nutrition of rabbits. J. Nutr. 69:28-32.

Topliff, D.R., M.A. Kennerly, D.W. Freeman, R.G. Teeter and D.G. Wagner. 1989. Changes in urinary and serum calcium and chloride concentrations in exercising horses fed varying cation-anion balances. Proc. Eleventh Equine Nutr. and Physio. Symp. Stillwater, OK. 1-2.

Tucker, W.B., G.A. Harrison and R.W. Hemken. 1988. Influence of dietary cation-anion balance on milk, blood, urine and rumen fluid in lactation dairy cattle. J. Dairy Sci. 71:346-354.

Tucker, W.B., J.F. Hogue, D.F. Waterman, T.S. Swenson, Z. Xin, R.W. Hemken, J.A. Jackson, G.D. Adams, and L.J. Spicer. 1991. Role of sulfur and chloride in the dietary cation-anion balance equation for lactating dairy cattle. J. Anim. Sci. 69:1205-1213.

Walser, M.S. and A.A. Browder. 1959. Ion association. II. The effect of sulfate infusion on calcium excretion. J. Clin. Invest. 38:1404-1411.

Whiting, S.J. and D.E. Cole. 1986. Effect of dietary cation-anion composition on acid-induced hypercalciuria in the adult rat. J. Nutr. 116:388-294.

Whiting, S.J. and H.H. Draper. 1980. The role of sulfate in the calciuria of high-protein diets in adult rats. J. Nutr. 110:212--222.

Williamson, H.M. 1974. Normal and abnormal electrolyte levels in the racing horse and their effect on performance. Jl. S. Afr. Vet. Ass. 45(4) 335-340.

Yen, J.T., W.G. Pond, and R.L. Prior. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52:778.

Young, J.K., G.D. Potter, L.W. Greene and J.W. Evans. 1989. Mineral balance in resting and exercised miniature horses. Proc. Eleventh Equine Nutr. and Physio. Symp. Stillwater, OK. 79-84.

APPENDIX

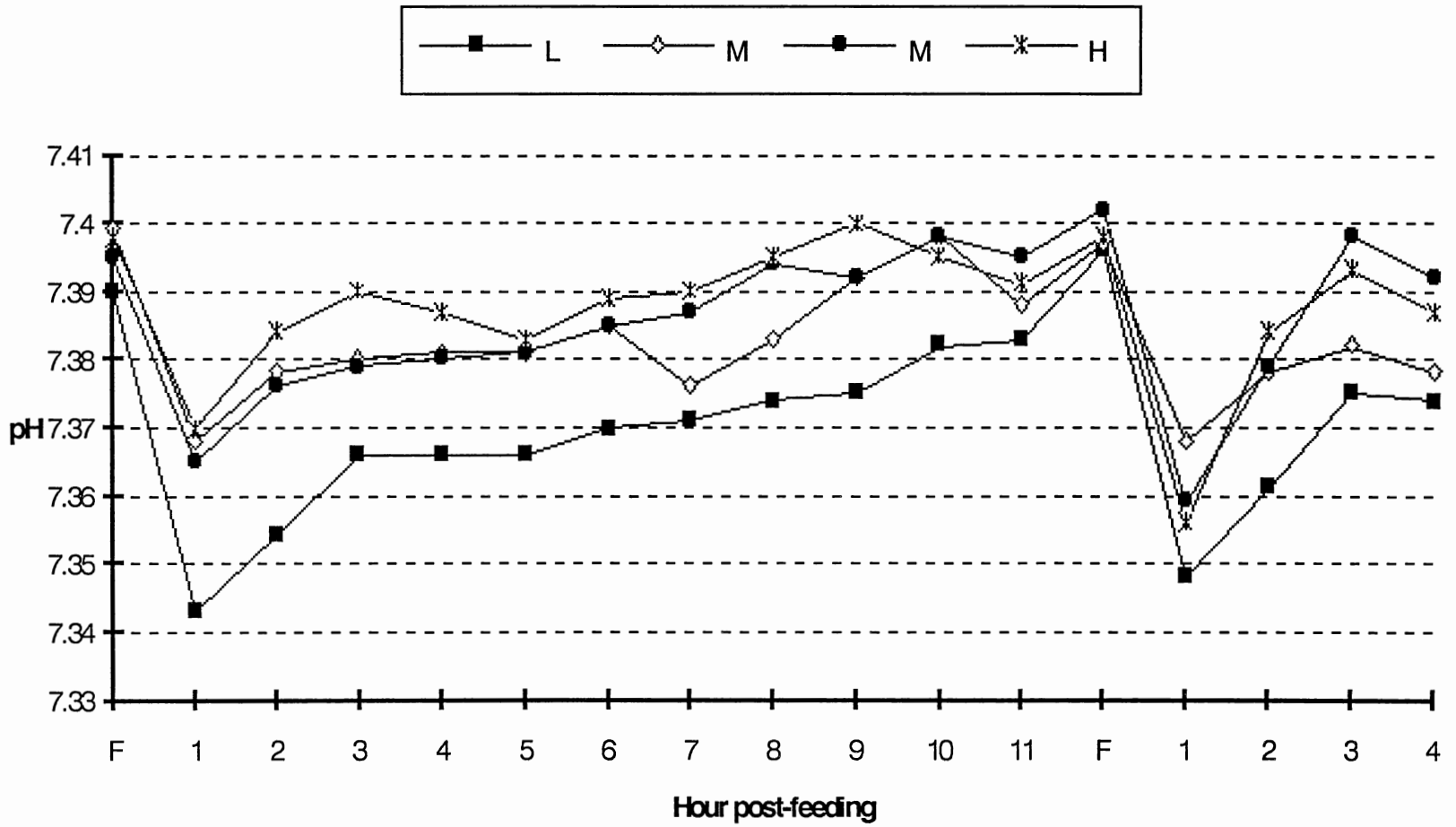

Figure 1. The Effect of Dietary Cation-Anion Balance on Venous Blood pH Post-Feeding in the Anaerobically Exercised Horse.

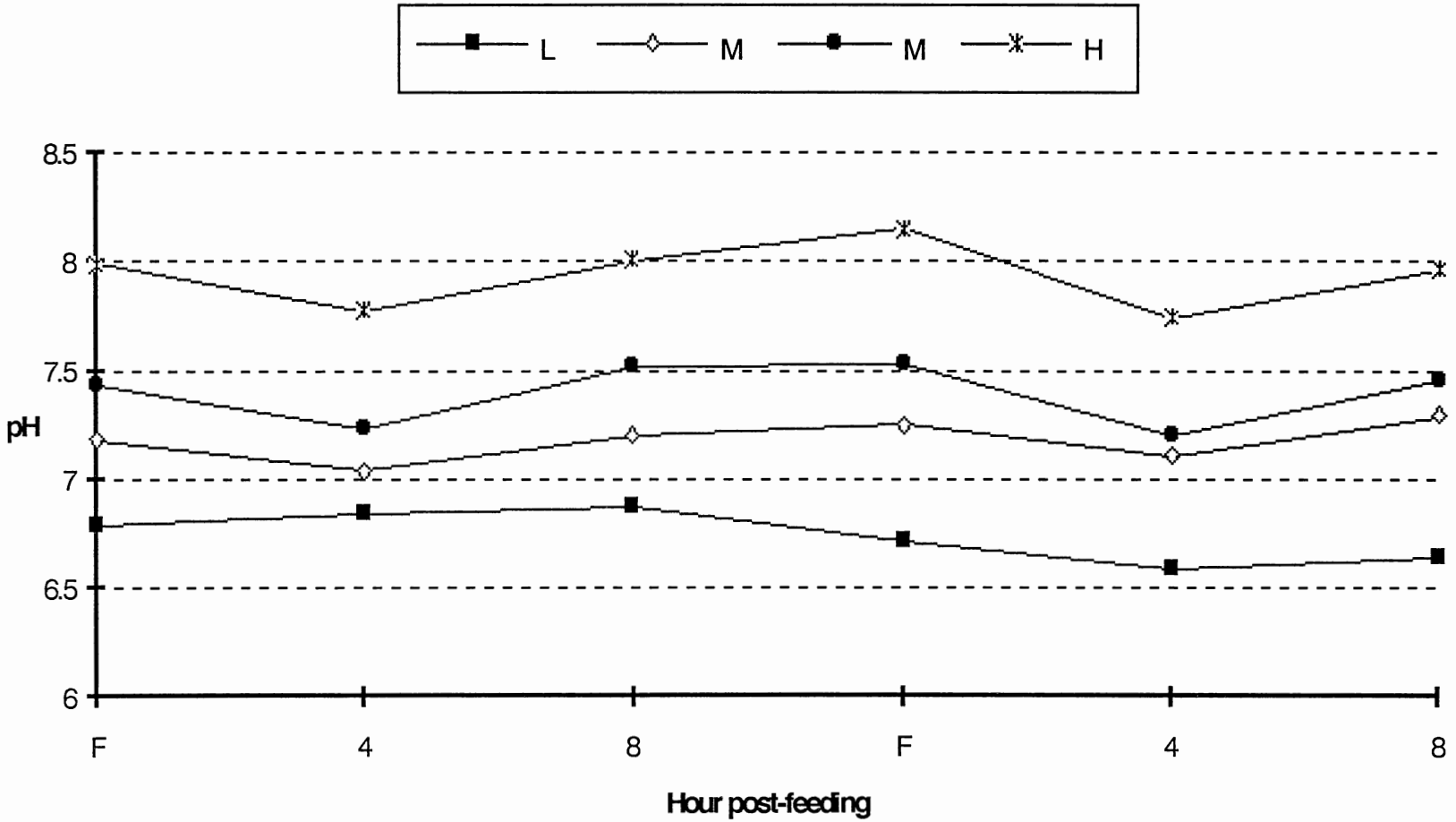

Figure 2. The Effect of Dietary Cation-Anion Balance on Urine pH Post-Feeding in the Anaerobically Exercised Horse.

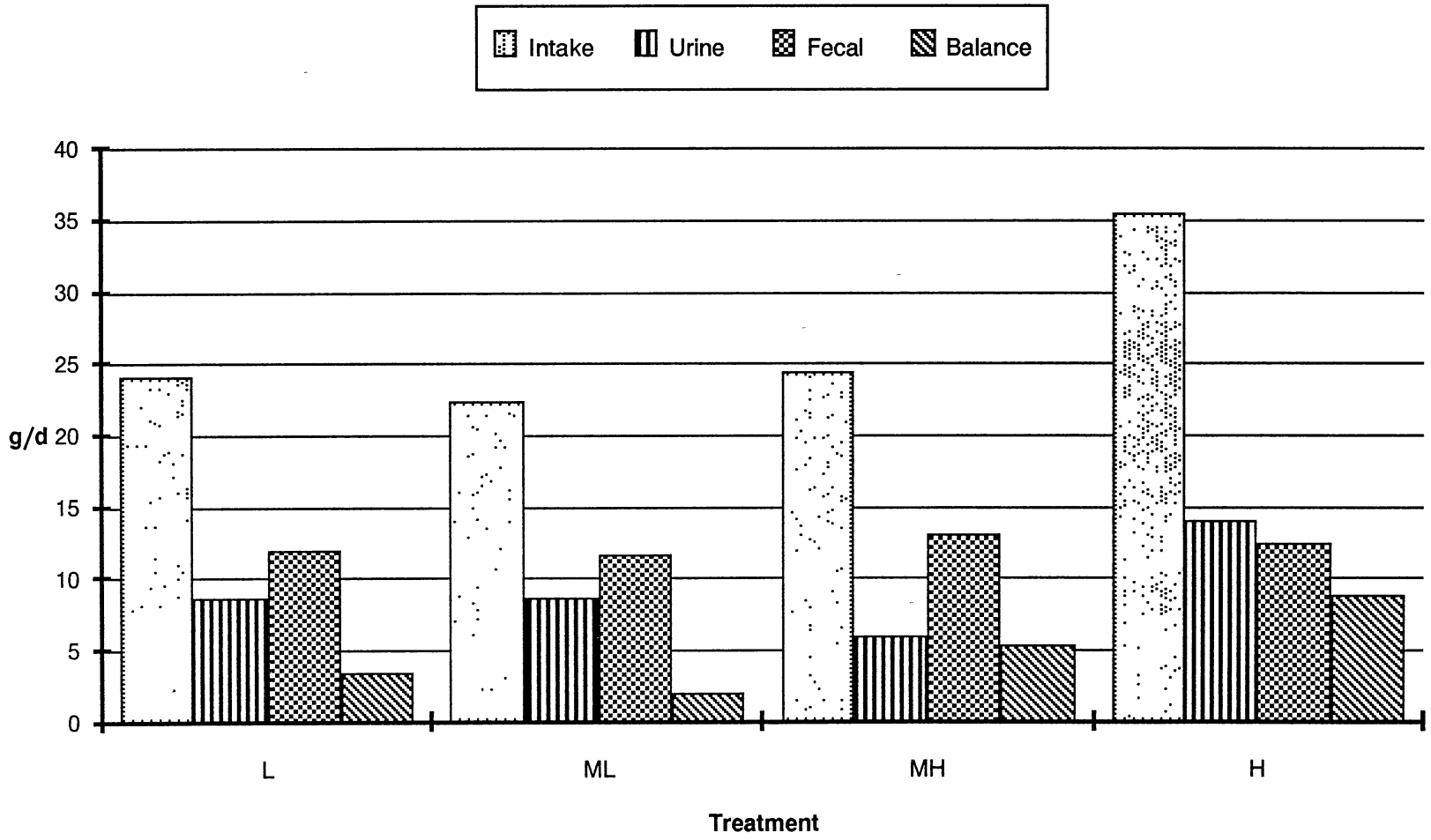

Figure 3. The Effect of Dietary Cation-Anion Balance on Sodium Balance in the Anaerobically Exercised Horse.

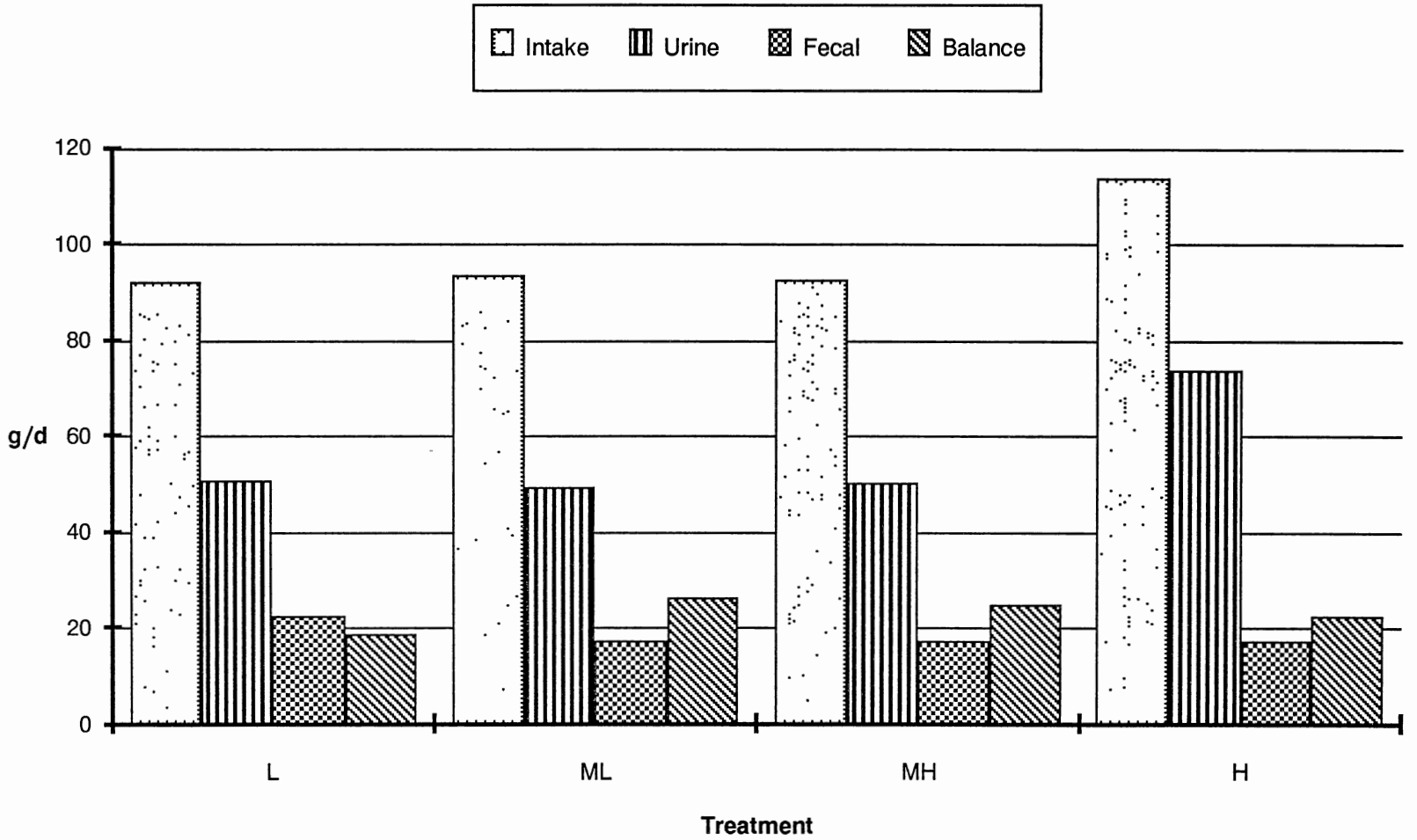

Figure 4. The Effect of Dietary Cation-Anion Balance on Potassium Balance in the Anaerobically Exercised Horse.

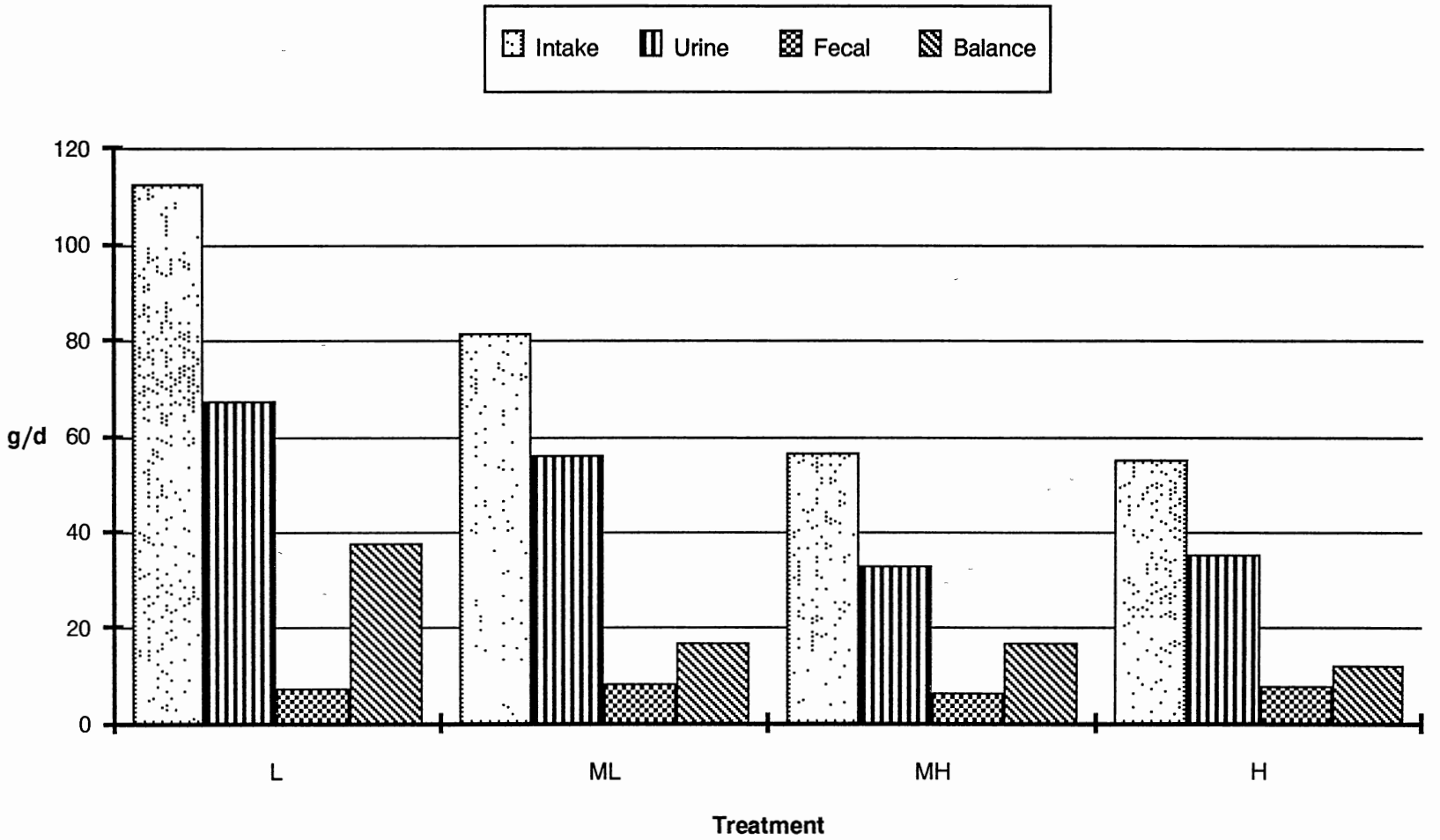

Figure 5. The Effect of Dietary Cation-Anion Balance on Chloride Balance in the Anaerobically Exercised Horse.

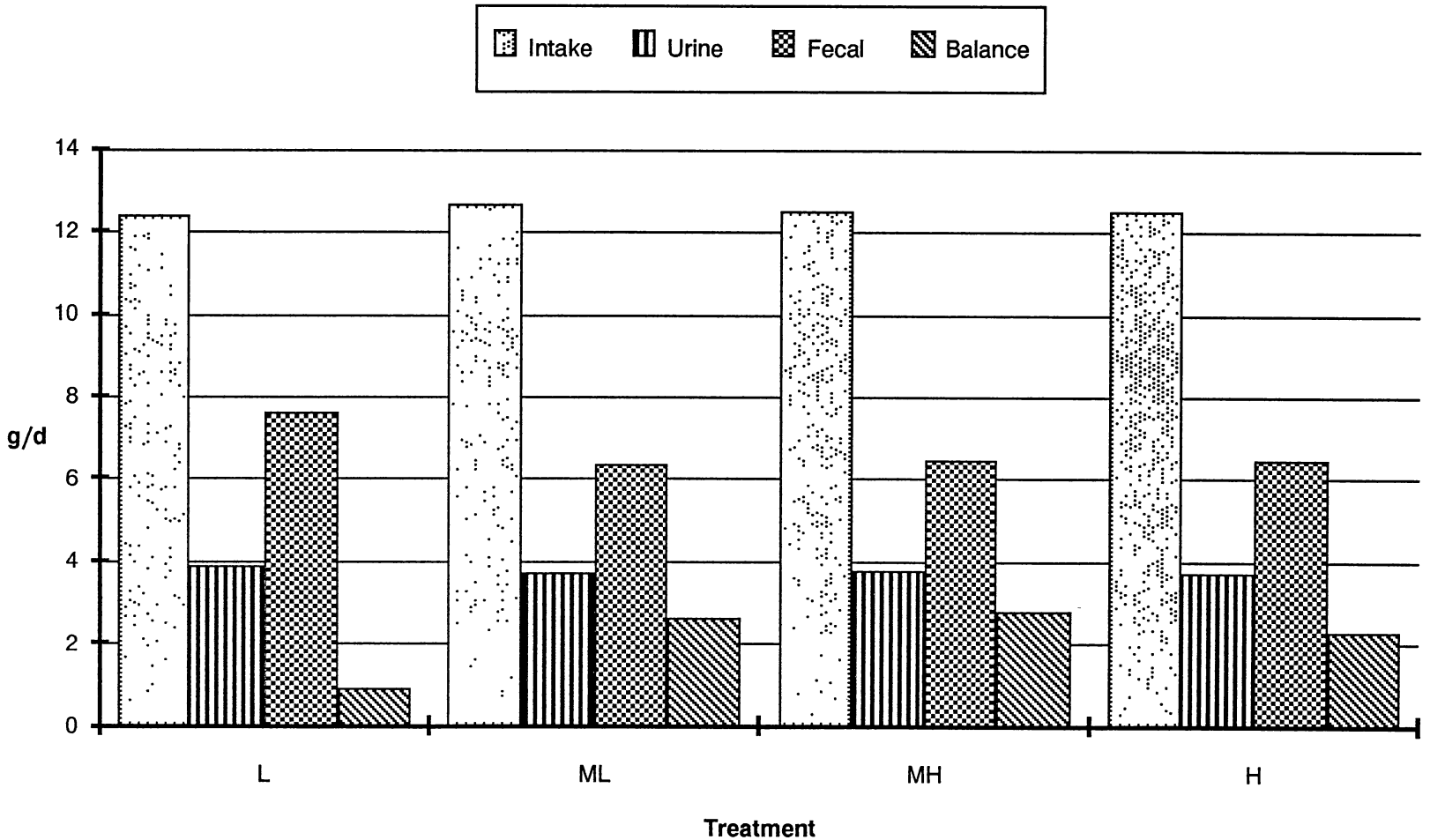

Figure 6. The Effect of Dietary Cation-Anion Balance on Magnesium Balance in the Anaerobically Exercised Horse.

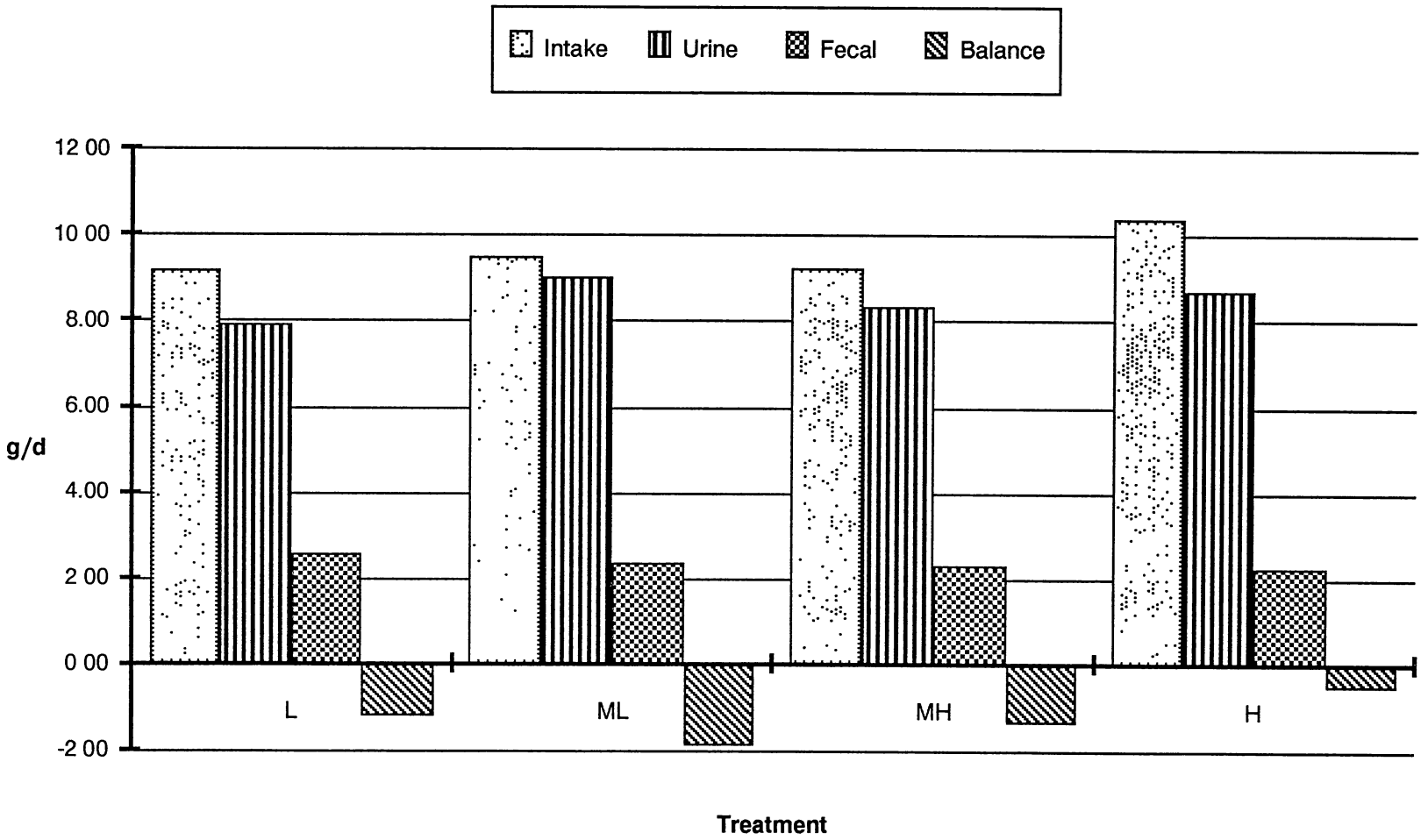

Figure 7. The Effect of Dietary Cation-Anion Balance on Sulfur Balance in the Anaerobically Exercised Horse.

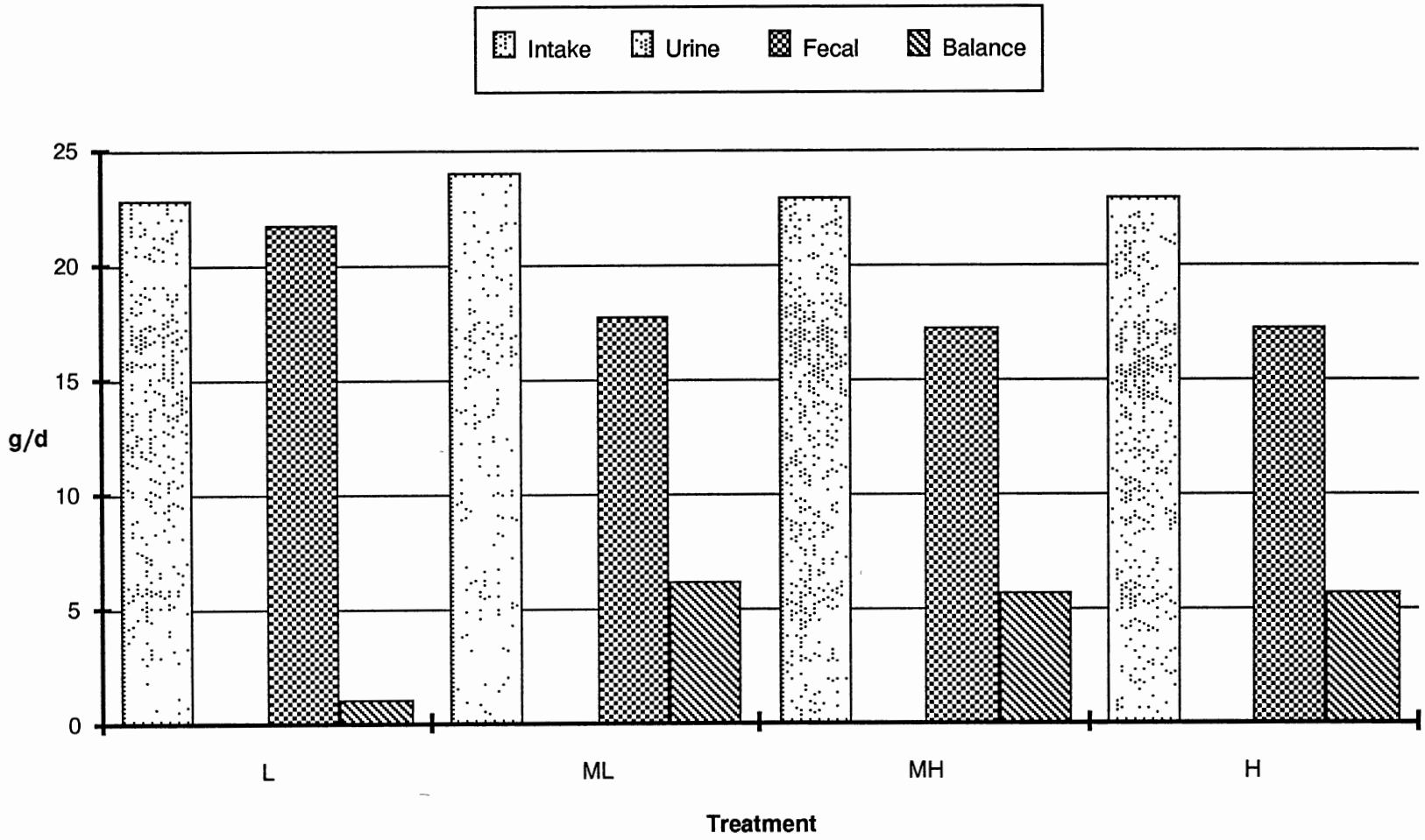

Figure 8. The Effect of Dietary Cation-Anion Balance on Phosphorus Balance in the Anaerobically Exercised Horse.

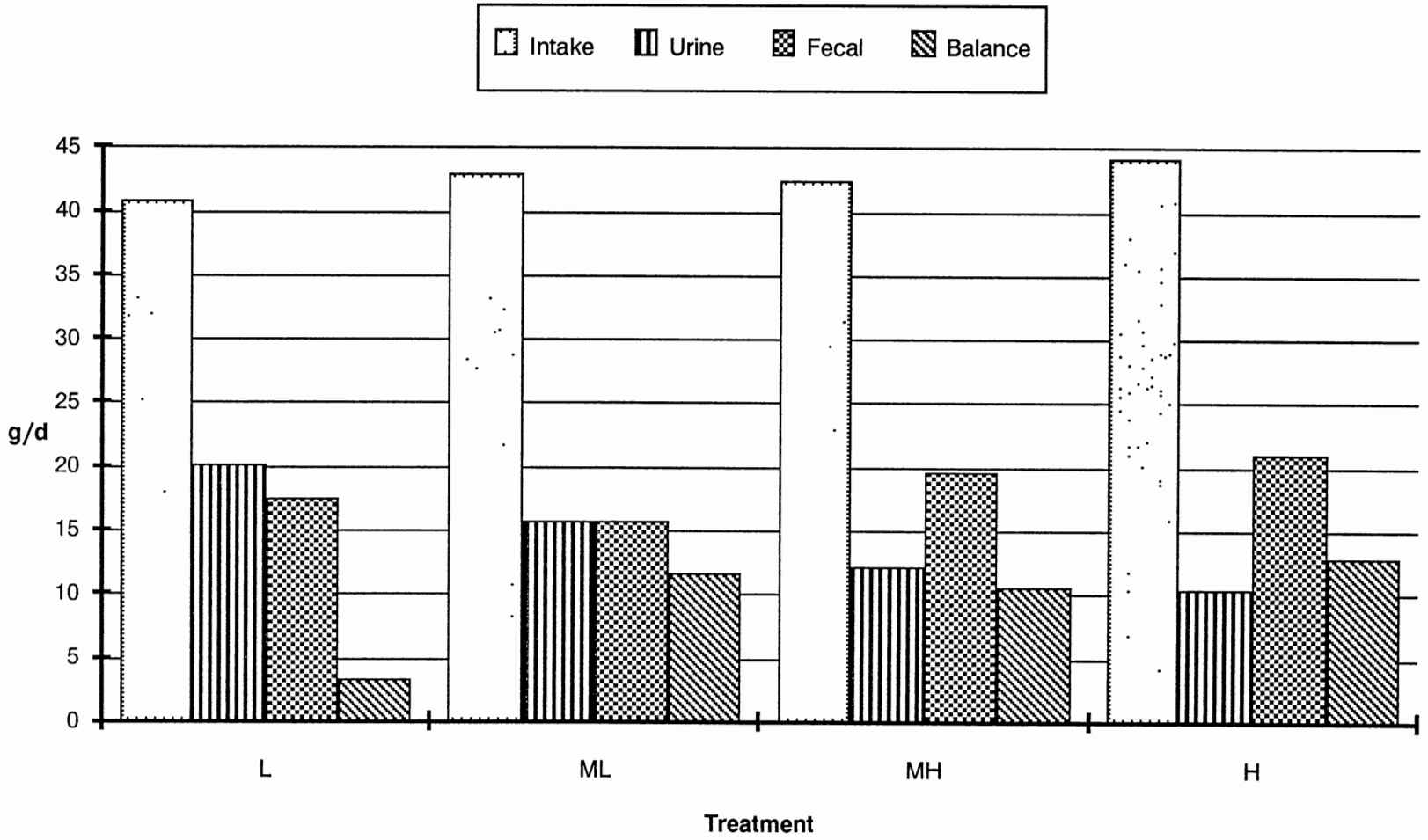

Figure 9. The Effect of Dietary Cation-Anion Balance on Calcium Balance in the Anaerobically Exercised Horse.

Vita

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Master of Science

Thesis: THE EFFECT OF DIETARY CATION-ANION BALANCE ON MINERAL BALANCE IN THE ANAEROBICALLY EXERCISED HORSE

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